Cellular homeostasis

Proliferation ↔ Arrest

Survival ↔ Apoptosis

Undifferentiated ↔ Differentiated

Cellular homeostasis

Proliferation ↔ Arrest

Survival ↔ Apoptosis

Undifferentiated ↔ Differentiated

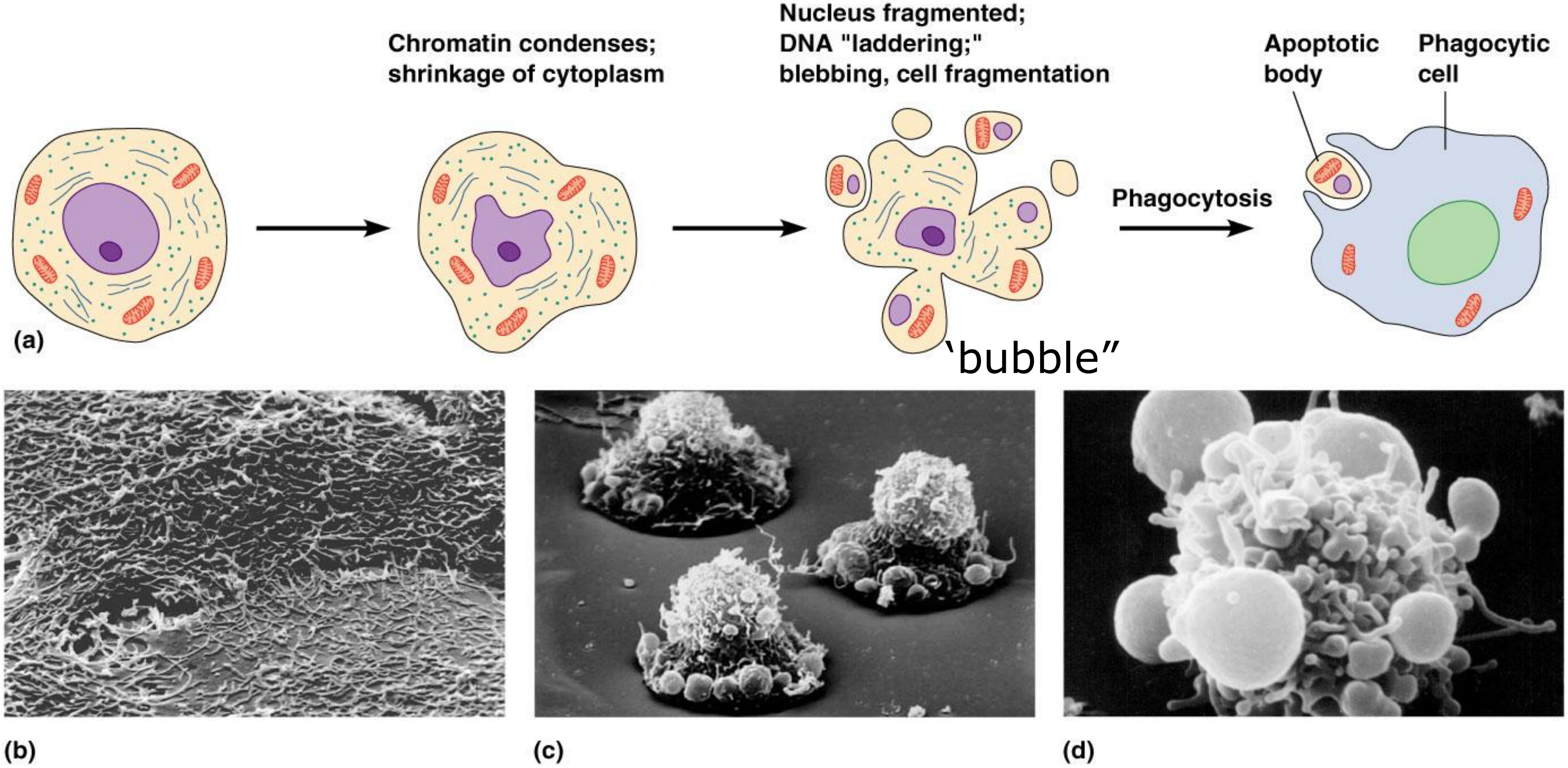
Cell Death

- The body is very good at maintaining a *constant number of cells*. So there has to exist mechanisms for ensuring other cells in the body are removed, when appropriate.
- Two forms
  - Apoptosis - suicide - programmed cell death
  - Necrosis - killing - decay and destruction

Стадии и признаки апоптоза

- Разрушение белков цитоскелета
- Раскрытие ядерных пор и разрушение ядра с последующим входом каспаз и активацией нуклеаз
- Конденсация и деградация хроматина (ДНК-лестница), инактивация репарации ДНК
- Изменения в ПлМ (потеря сиаловой кислоты, рецепторы к фибронектину, выведение ФС на поверхность)
- Фрагментация клетки с образованием апоптозных телец

Major Steps in Apoptosis


Chromatin condenses; shrinkage of cytoplasm
Nucleus fragmented; DNA "laddering;" blebbing, cell fragmentation
Apoptotic body
Phagocytic cell
Phagocytosis
(a)
(b)
(c)
(d)

`bubble”

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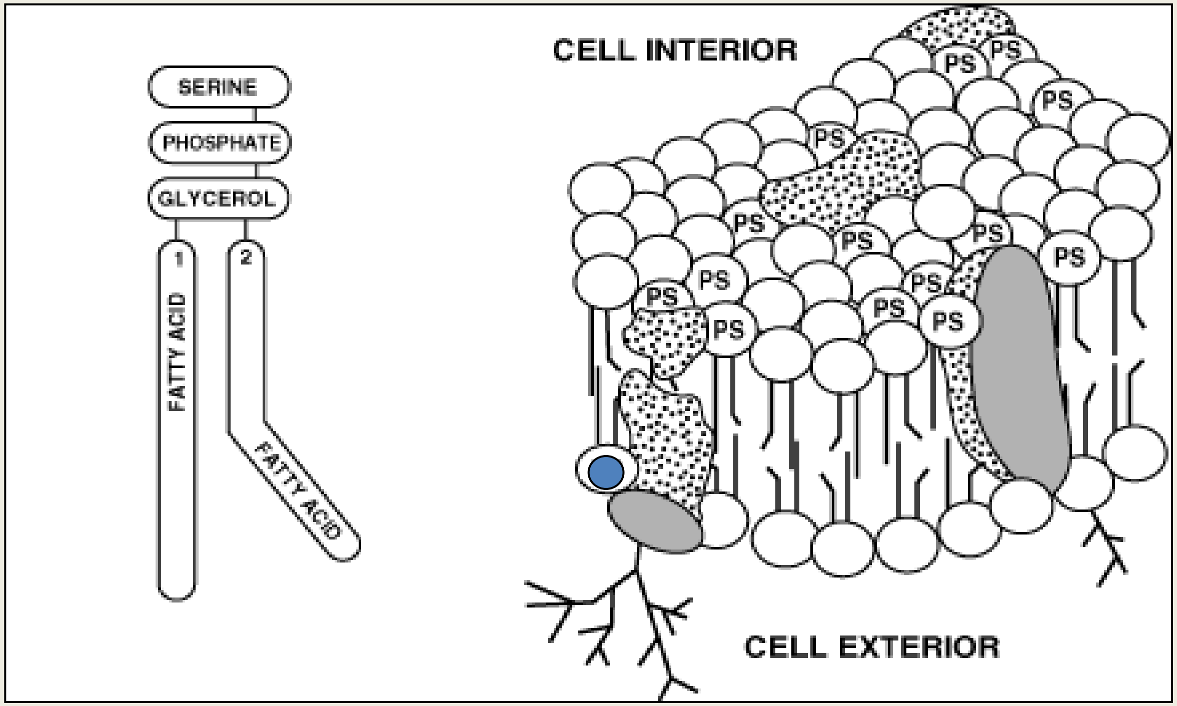
Necrosis= injury

Apoptosis= program for cell death

Necrosis *vs.* Apoptosis

Necrosis	**Apoptosis**
Cellular swelling	Cellular condensation
Membranes are broken	Membranes remain intact
ATP is depleted	Requires ATP
Cell lyses, eliciting an inflammatory reaction	Cell is phagocytosed, no tissue reaction
DNA fragmentation is random, or smeared	Ladder-like DNA fragmentation
In vivo, whole areas of the tissue are affected	In vivo, individual cells appear affected

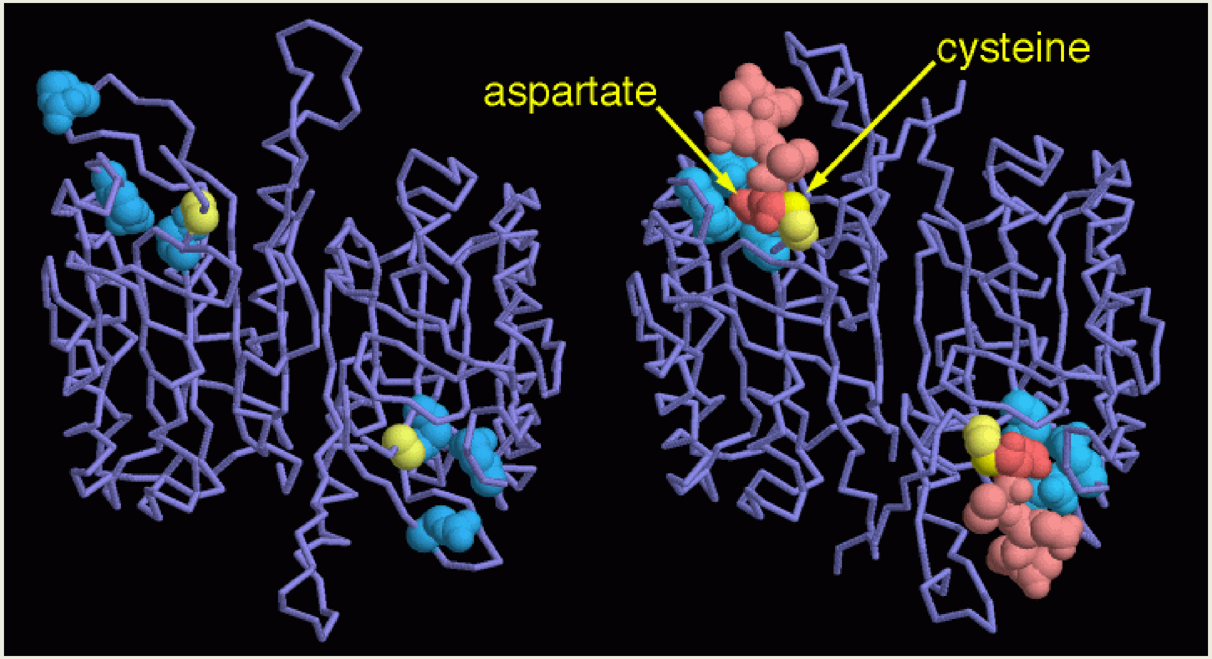
Cell removal



- Enzyme system keeps PS on inner surface
- Inhibited during apoptosis
- PS redistributed to extra-cellular surface

- Macrophage receptors recognise and bind PS
- Phagocytosis of apoptosome
- Release of anti-inflammatory substances

Каспазы и апоптоз

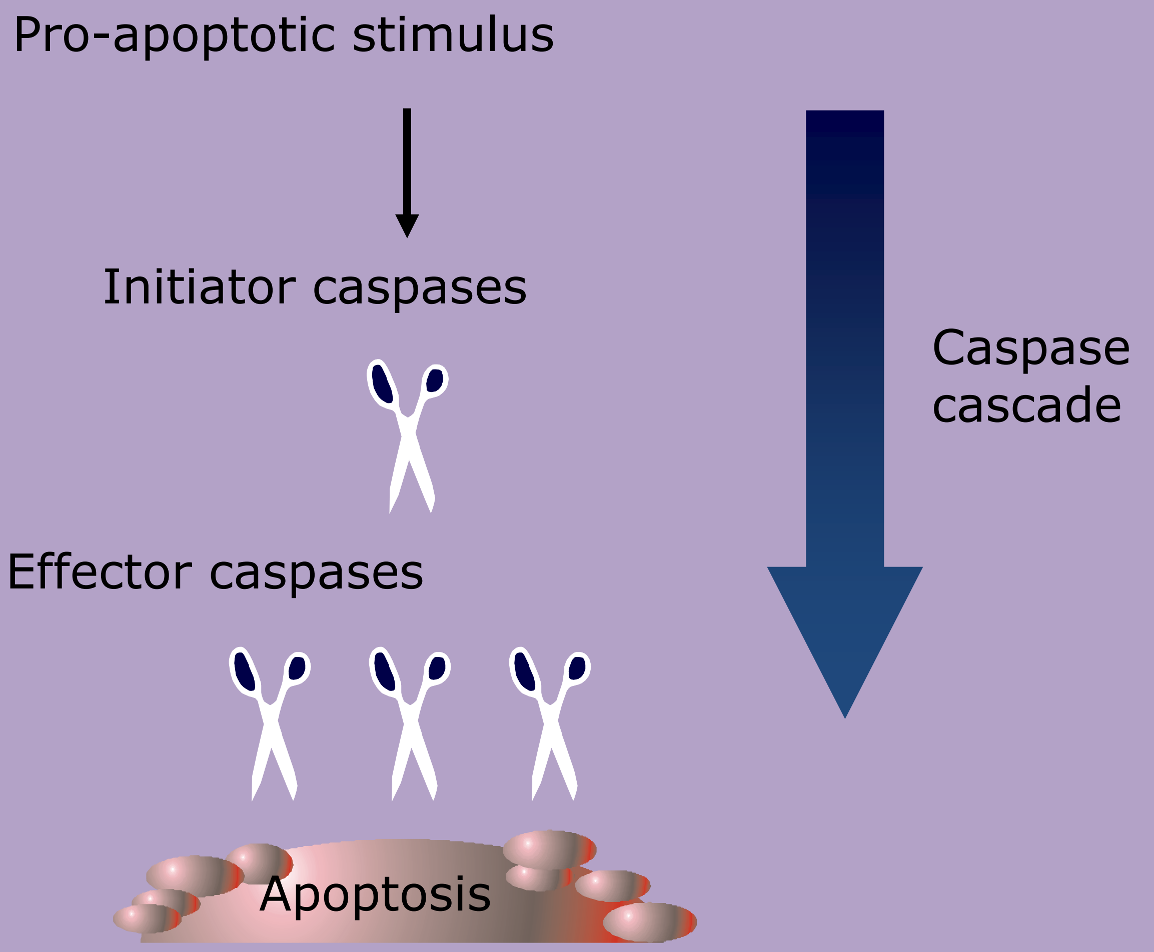


Caspases, or *c*ysteine-*asp*artic prote*ases* or **c**ysteine-dependent **asp**artate-directed prote**ases** are a family of cysteine proteases. Made as inactive precursors - procaspases

Типы каспаз

- **initiator (apical) caspases** (cleave inactive pro-forms of effector caspases, thereby activating them)
- **effector (executioner) caspases** (cleave other protein substrates within the cell, to trigger the apoptotic process).
- The initiation of this cascade reaction is regulated by caspase inhibitors.

Apoptosis is Carried out by Caspases



Pro-apoptotic stimulus

Initiator caspases

Effector caspases

Apoptosis

Caspase
cascade

Adapted from Thornberry NA, Lazebnik Y. Science 1998;281:1312–1316.
Caspase, cysteine aspartase.

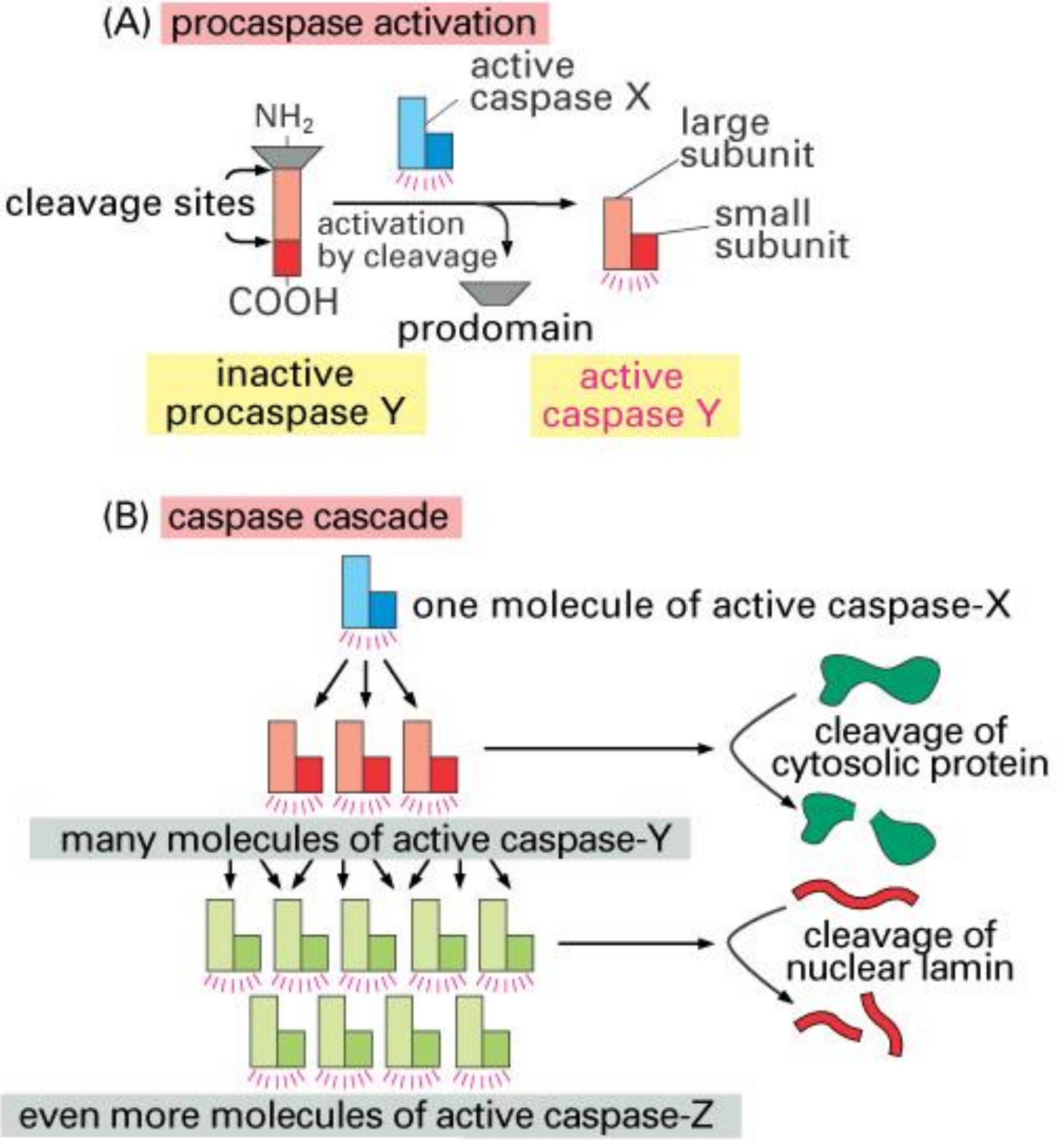

(A) procaspase activation
active caspase X
NH$_2$
large subunit
cleavage sites
activation by cleavage
small subunit
COOH
prodomain
inactive procaspase Y
active caspase Y
(B) caspase cascade
one molecule of active caspase-X
cleavage of cytosolic protein
many molecules of active caspase-Y
cleavage of nuclear lamin
even more molecules of active caspase-Z

Figure 18-21 Essential Cell Biology, 2/e. (© 2004 Garland Science)

Some cellular targets of caspases

FAK (focal adhesion kinase): inactivation of FAK disrupt cell adhesion, leading to detachment of the apoptotic cell from its neighbors.

Lamins: important component of the nuclear envelope. Cleavage of lamins leads to disassembly of the nuclear lamina.

Proteins required for cell structure: actin, intermediate filaments, etc. Cleavage of these proteins lead to changes in cell shape and the surface blebbing.

Endonuclease CAD: responsible for chromosome fragmentation. CAD cuts DNA into small fragments. CAD normally binds to an inhibitor protein. Caspases cleaves the inhibitor protein and activates CAD.

Enzymes involved in DNA repair: no need to repair DNA in cells destined to death.

Apoptosis signaling

2 осн. Пути *активации* апоптоза:

- Death activator or extrinsic pathway
  Внешний путь через семейство DR
- Mitochondrial or intrinsic pathway
  Внутренний путь через митохонриальные компоненты и выход цитохрома с

The Extrinsic Apoptosis Pathway

- The extrinsic pathway triggers apoptosis in response to the activation of **pro-apoptotic receptors**, such as DR4 and DR5, by specific **pro-apoptotic ligands**, such as Apo2L/TRAIL

- This pathway stimulates apoptosis **independently of p53**

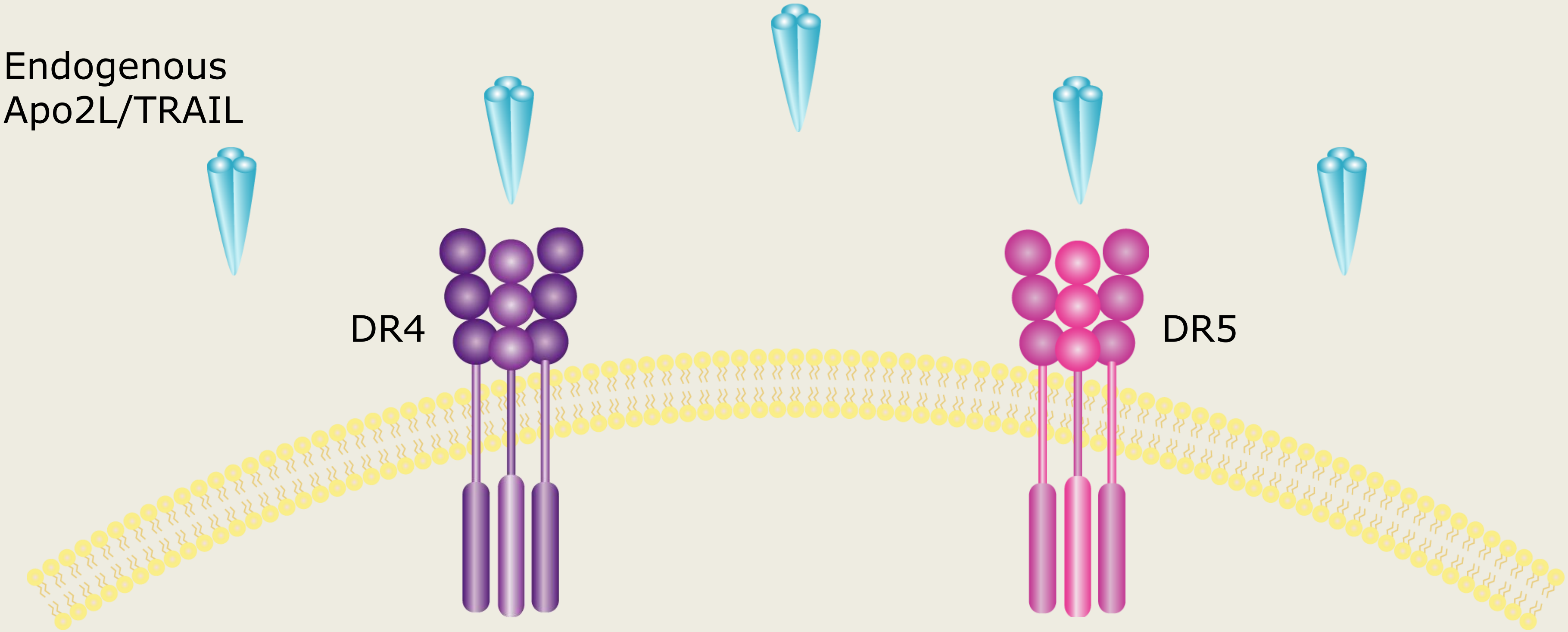
- Ligand-induced activation of DR4 and DR5 leads to the rapid assembly of the death-inducing signaling complex (**DISC**) and the recruitment of initiator caspases 8 and 10 through the adaptor Fas-associated death domain (**FADD)**

- Caspases 8 and 10 activate effector caspases 3, 6, and 7, leading to apoptosis

Рецепторы на ПлМ: роль в сигналинге при апоптозе

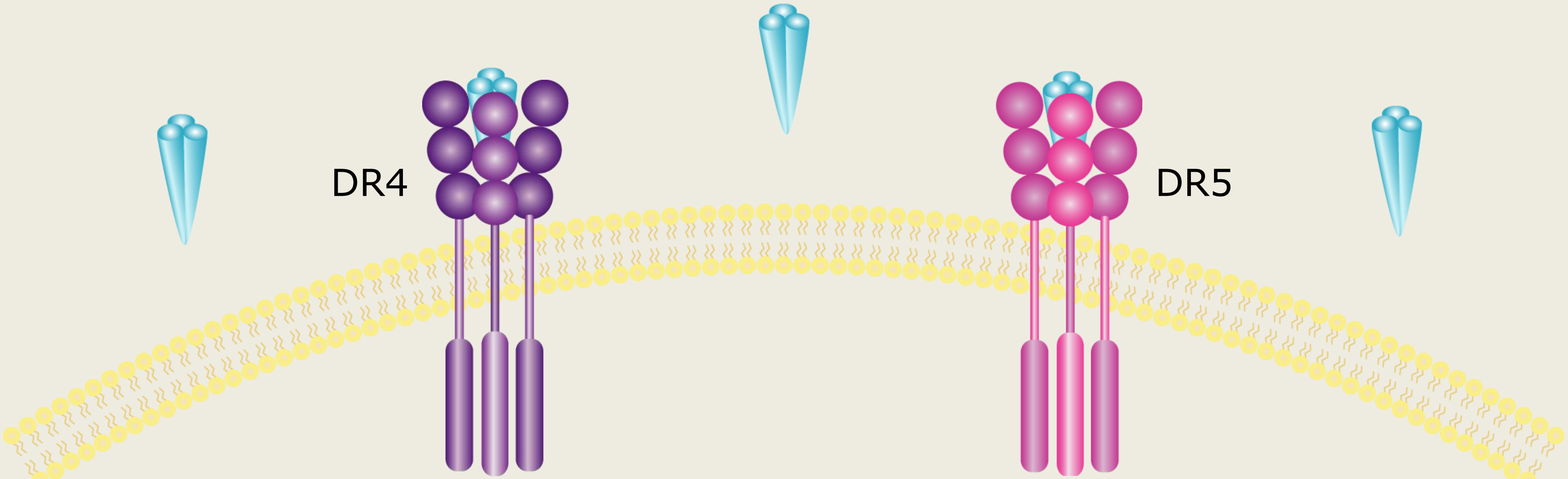
- DR (death receptors)
- Fas/Fas ligand (FasL)
- TRAILR

Apoptosis via Pro-apoptotic Receptors (DR4 and DR5)



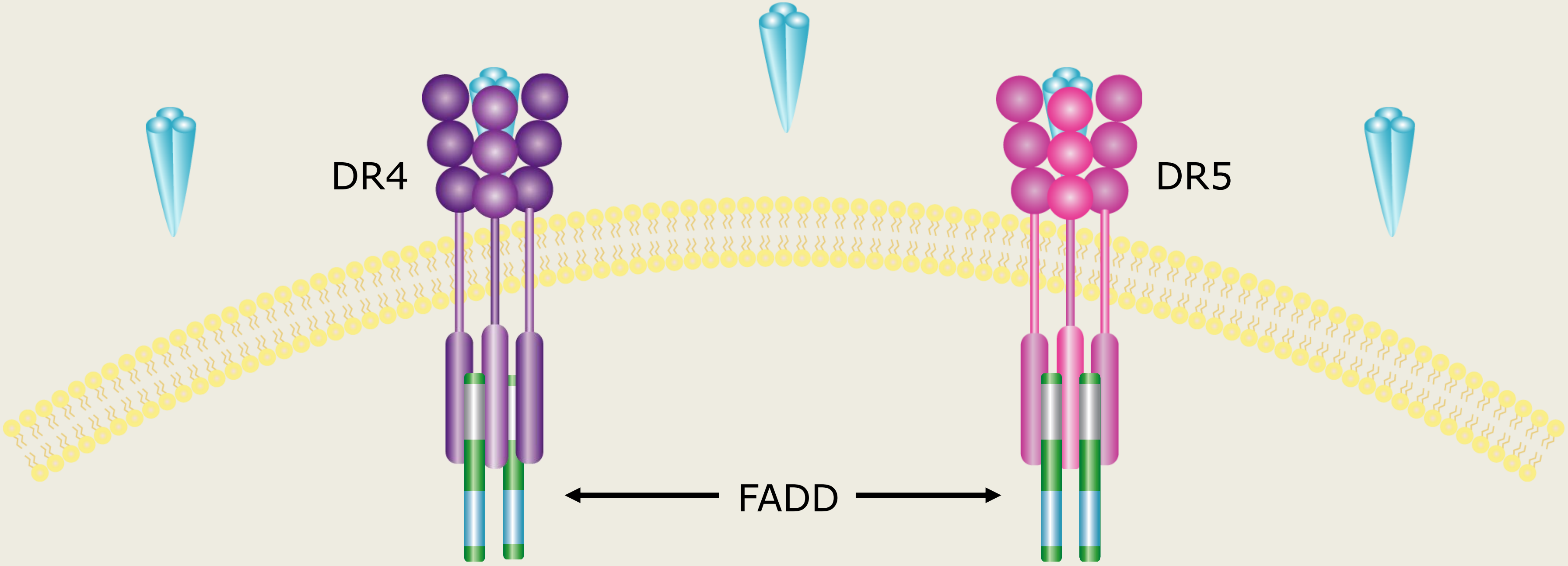
Apo2L/TRAIL, apoptosis-inducing ligand 2/tumor necrosis factor-related apoptosis-inducing ligand; DR, death receptor

Endogenous Apo2L/TRAIL Activates Pro-apoptotic Receptors (DR4 and DR5)



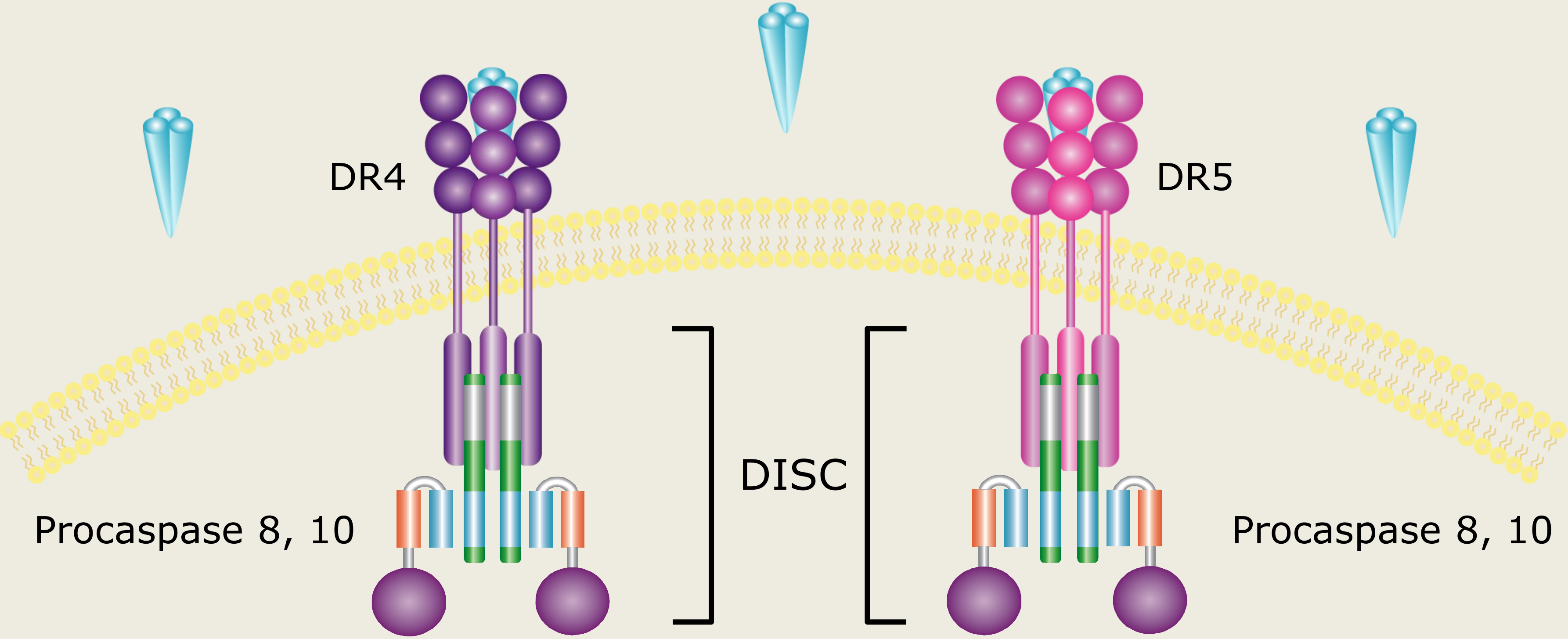
DR, death receptor.

Pro-apoptotic Receptors (DR4 and/or DR5) Recruit FADD



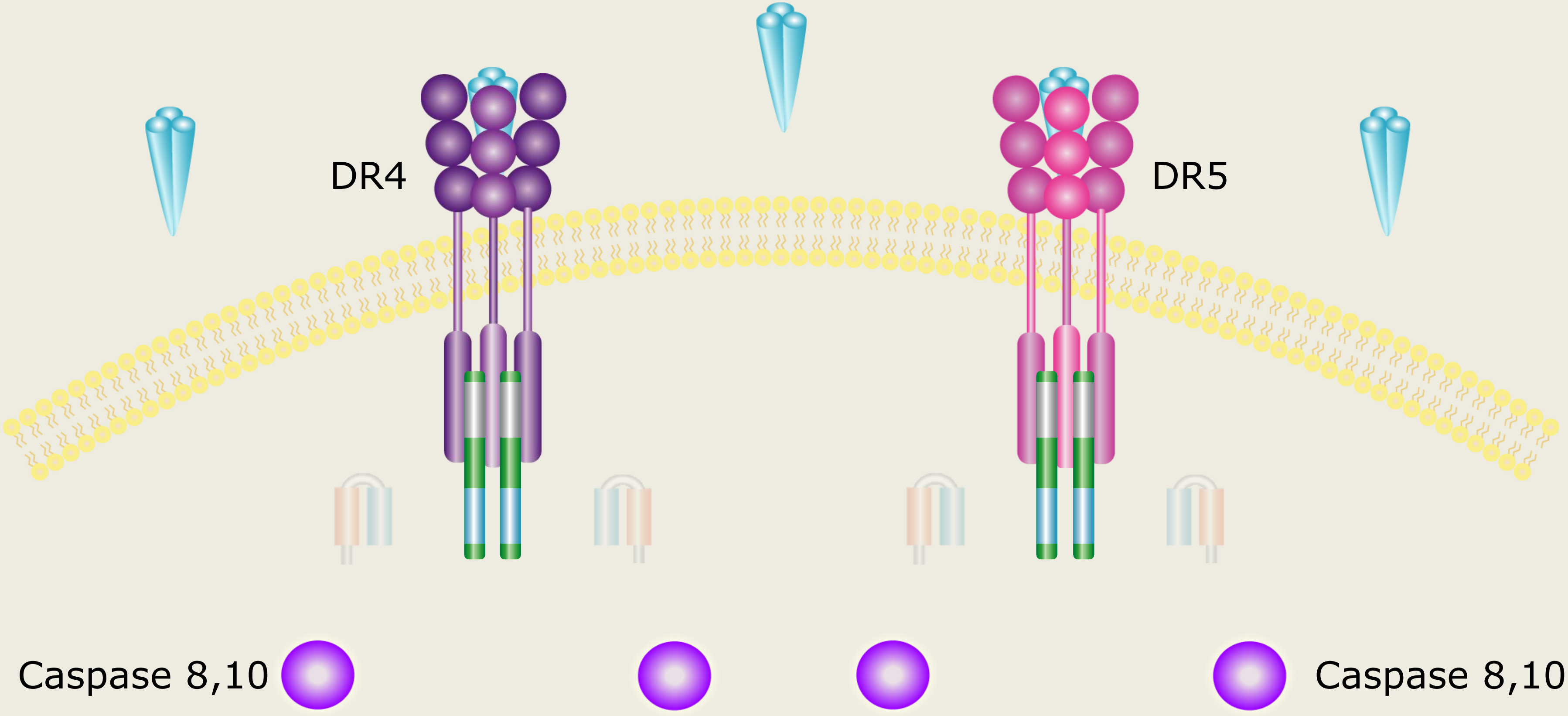
DR, death receptor; FADD, Fas-associated death domain.

FADD Recruits Initiator Caspase 8 and/or 10 to the DISC



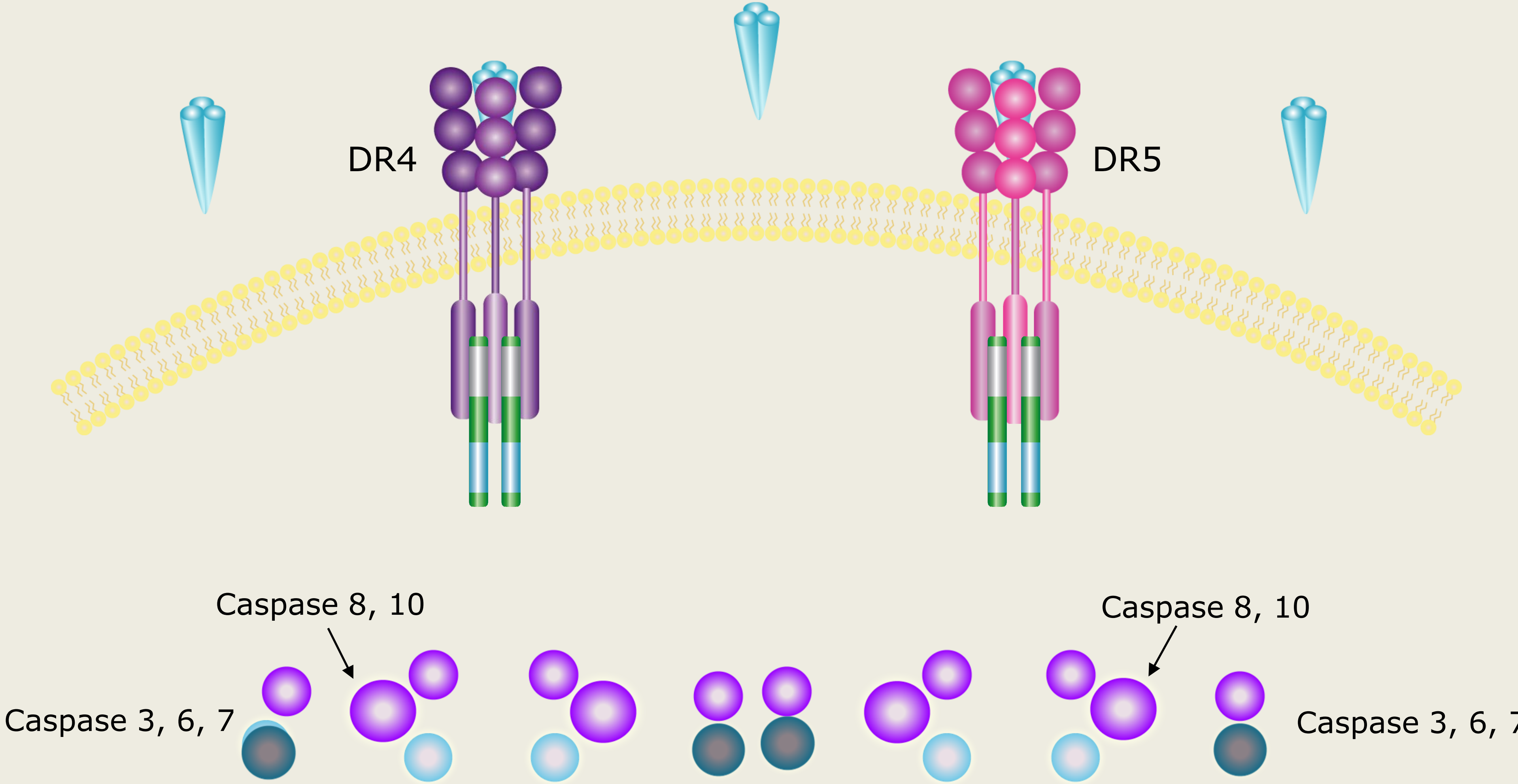
DR, death receptor; DISC, death-inducing signaling complex.

Activated Caspase 8 and 10 are Released Into the Cytoplasm



DR, death receptor.

Caspase 3, 6, and 7 are Activated



DR, death receptor.

Summary of death receptor pathway

ligands

↓

Death receptors

↓

FADD/DISC

↓

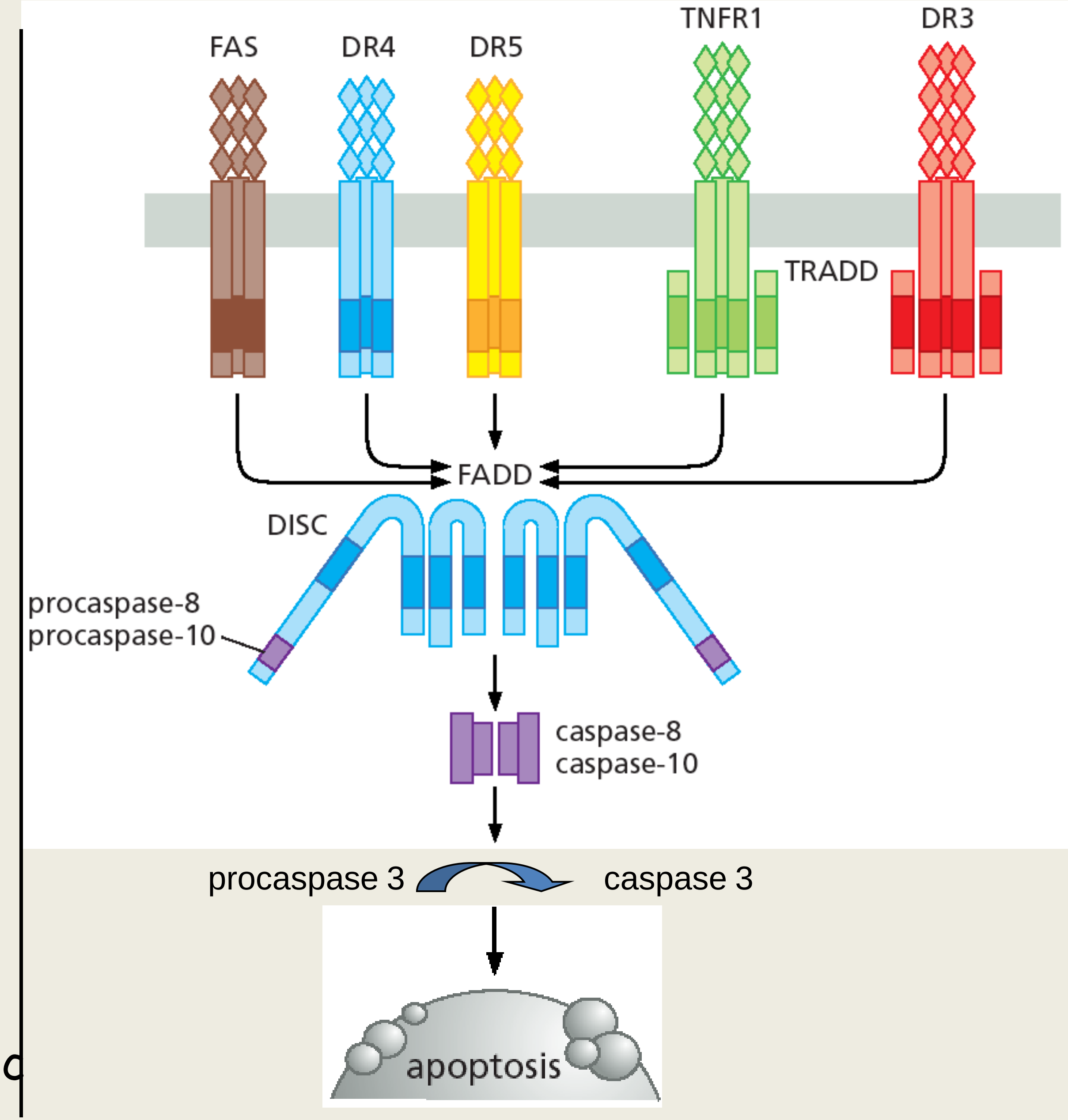
Caspase 8, 10

↓

Caspase 3, 6, 7

↓

Lamin, CAD,actin,
Adhesion molecules, etc



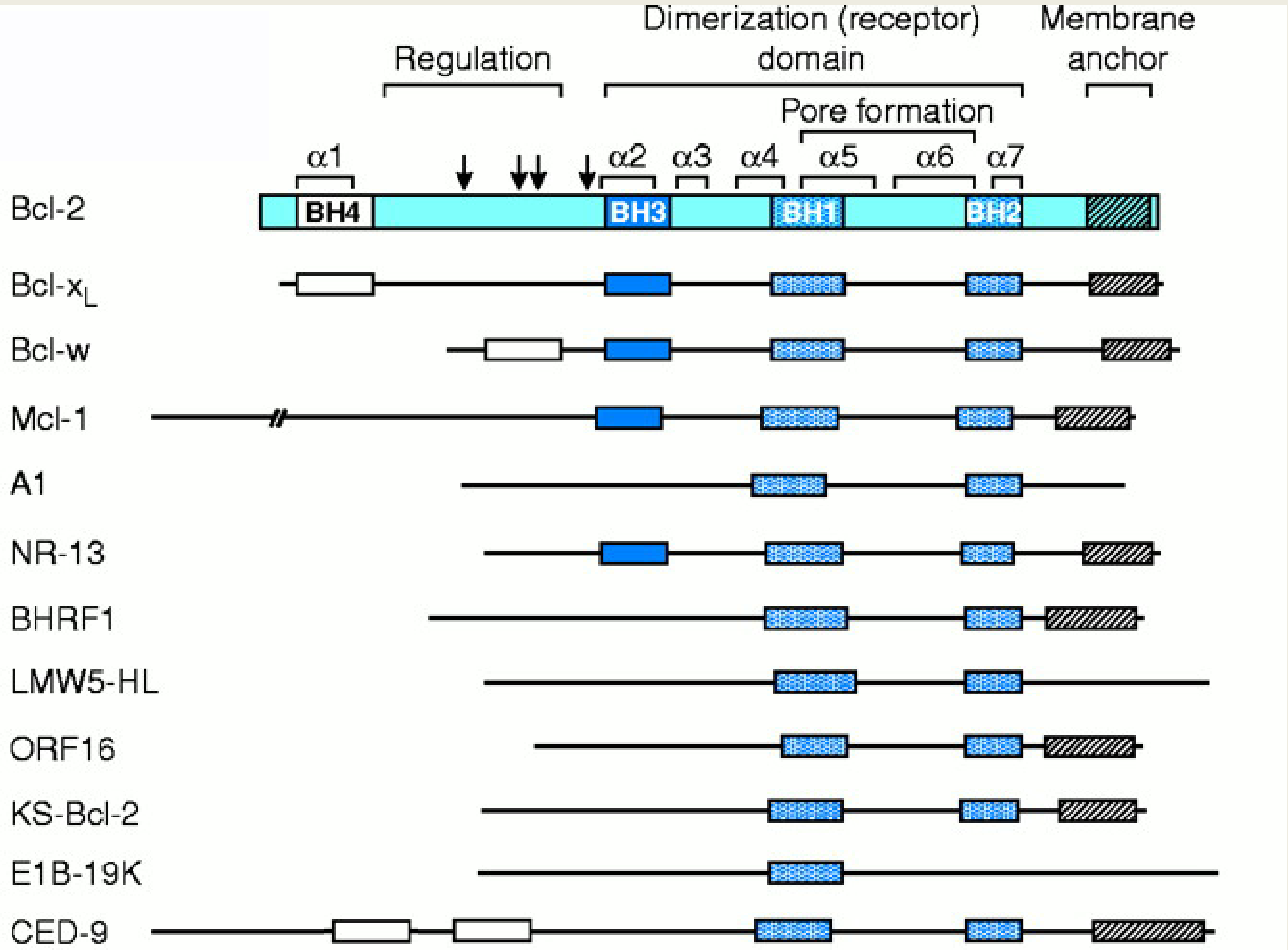
The Intrinsic Apoptosis Pathway

- The intrinsic pathway is triggered by the **p53 tumor-suppressor** in response to DNA damage and other types of severe cell stress
- Conventional anticancer therapies, such as chemotherapy and radiotherapy, activate this pathway via p53
- p53 activates the intrinsic pathway through transcriptional upregulation of **pro-apoptotic members** of the BCL2 family of proteins such as PUMA and BAX
- BAX causes the release of **cytochrome *c* from the mitochondria**, which together with the adaptor APAF1, activate the initiator caspase 9
- Caspase 9 activates the effector caspases 3, 6, and 7, which are responsible for destroying critical components of the cell, and inducing apoptosis
- p53 is inactivated by mutations in more than half of human cancers

Bcl-2 proteins

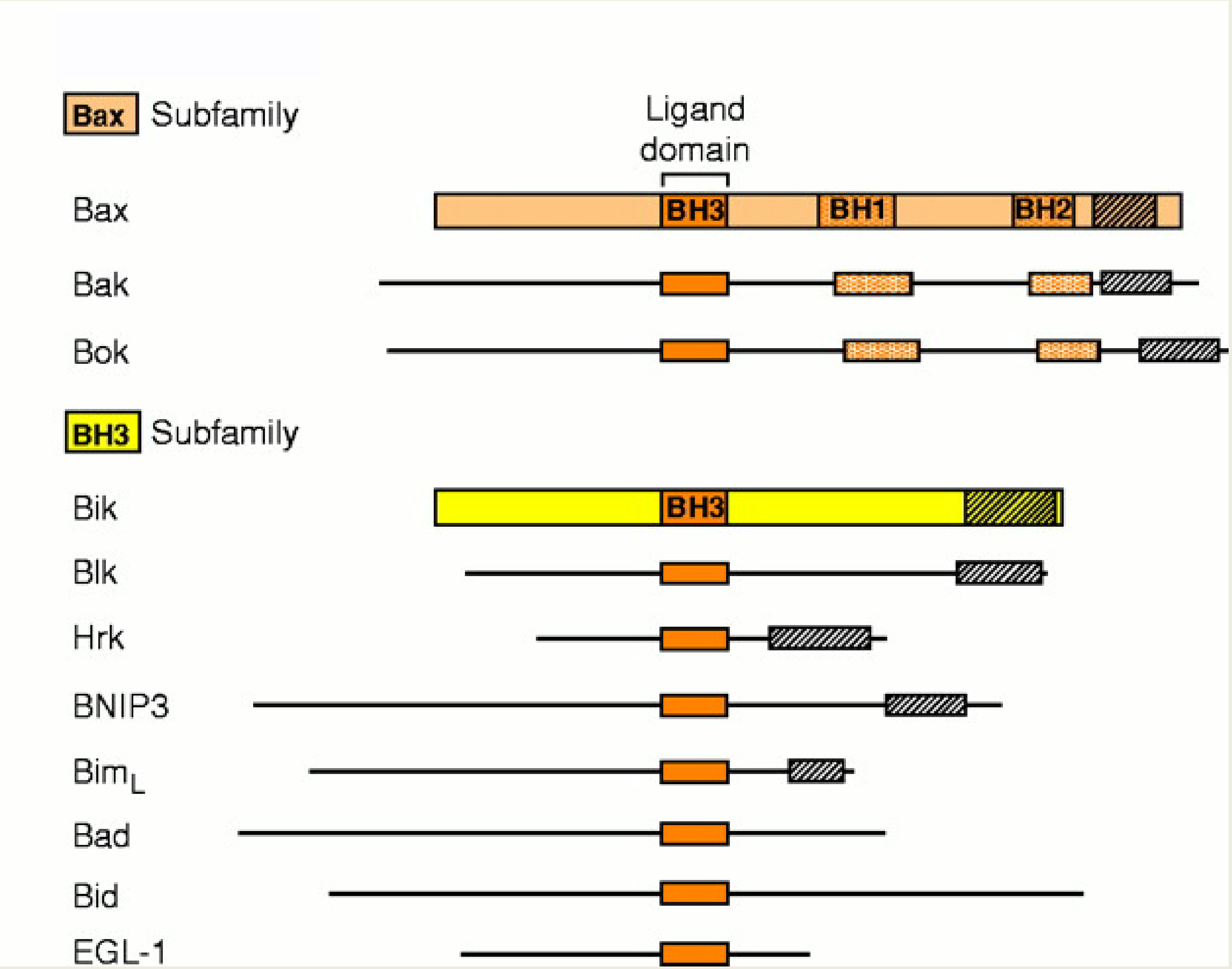
- Large family of proteins
- Named from B cell lymphoma
- Some are pro-apoptotic some are anti-apoptotic

Anti-apoptotic Bcl-2 family members

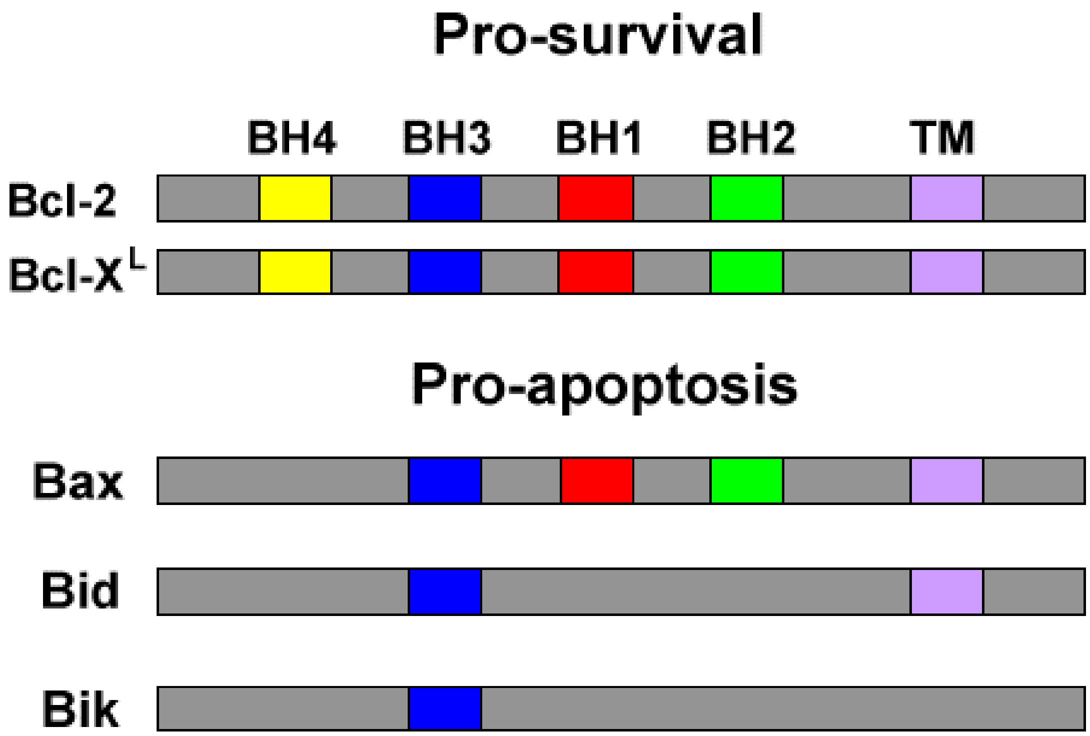


Adapted from Adams and Cory, Science 281, 1322-1226, 1998

Pro-apoptotic Bcl-2 family protein



Adapted from Adams and Cory, Science 281, 1322-1



Pro-survival

BH4 BH3 BH1 BH2 TM

Bcl-2

Bcl-X^{L}

Pro-apoptosis

Bax

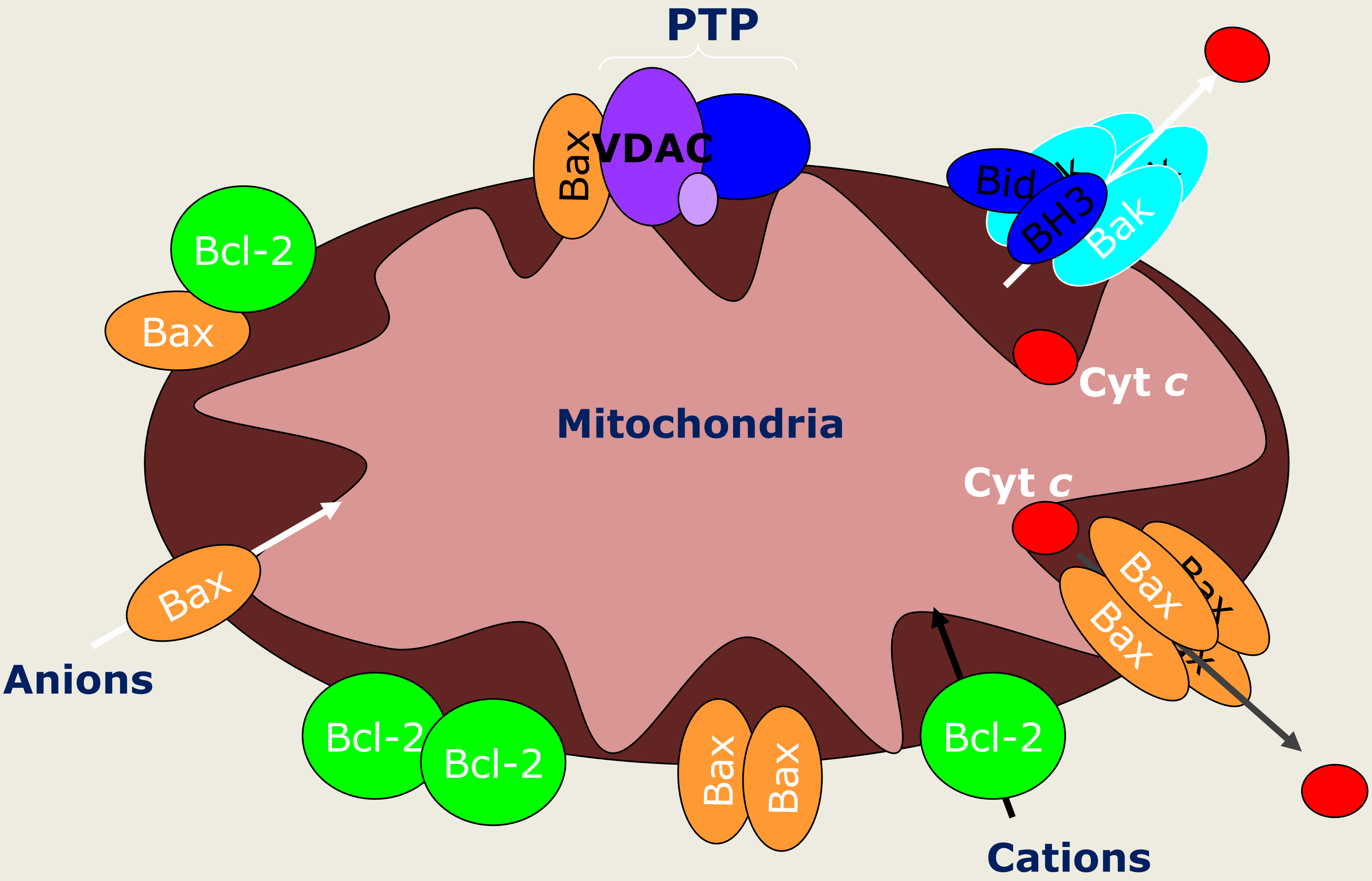
Bid

Bik

Key Features of Bcl-2 Family Proteins

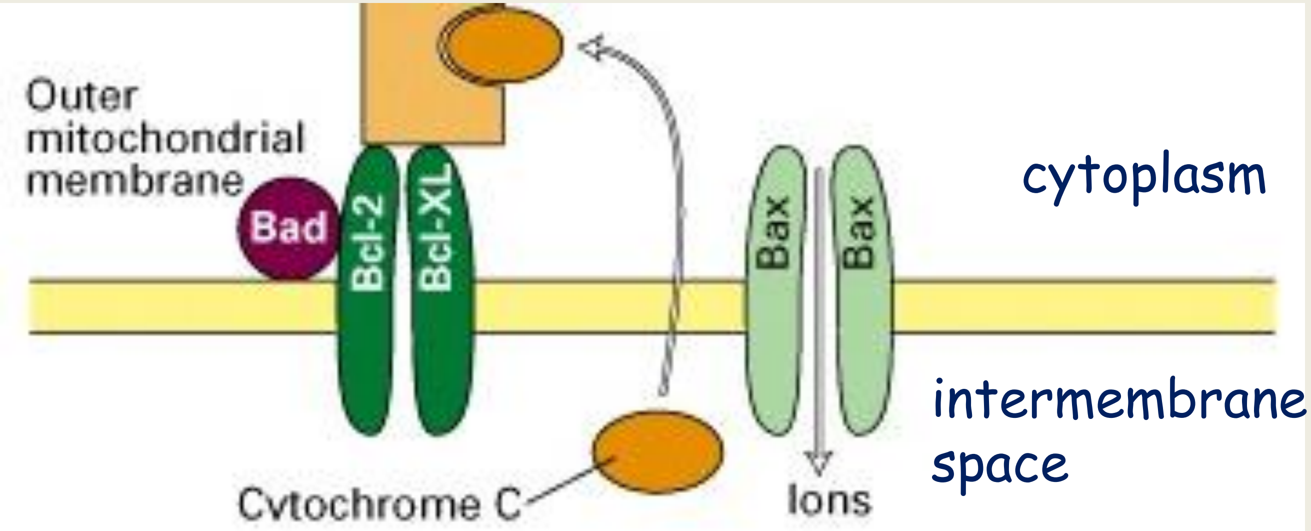
- They localize either inducibly or constitutively to outer membranes of mitochondria and nuclei, and membranes of ER
- They are capable of forming heterodimers with other family members, especially those with an amphipathic helical BH3 domain
- They are capable of forming ion-conducting channels on synthetic membranes

Bcl-2 Family Member Regulation of the Mitochondria



Bcl-2 family proteins control cytochrome c translocation

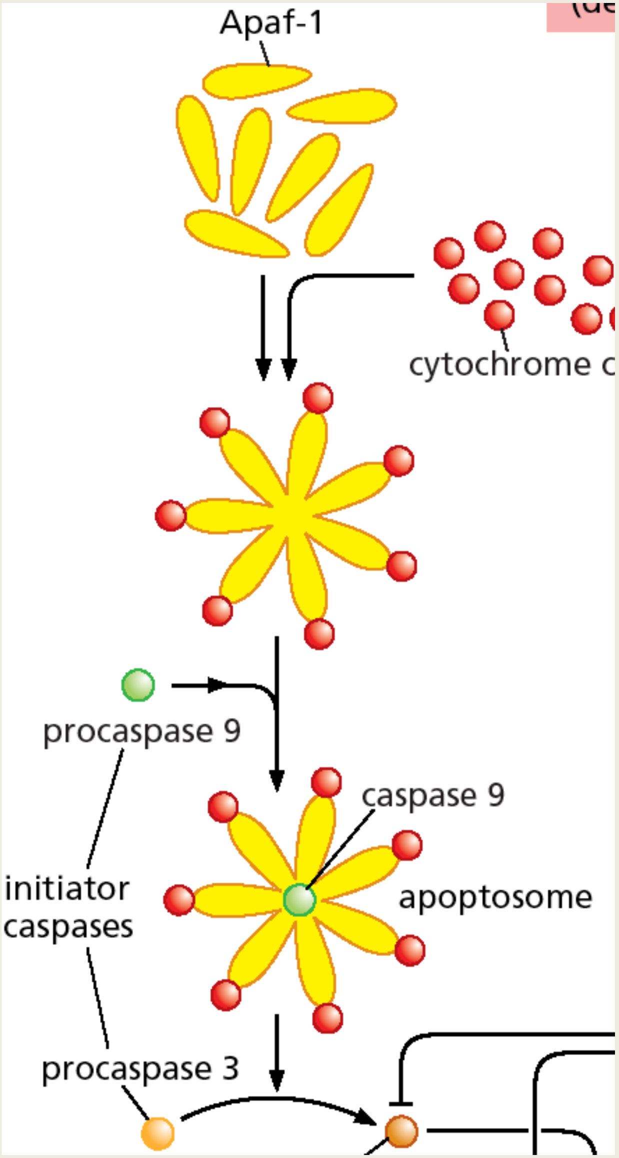
- **Bax** forms homo-dimers in the presence of apoptotic signals. This homodimer presumably promotes the opening of a channel that controls the translocation of cytochrome c from the intermembrane space to cytoplasm



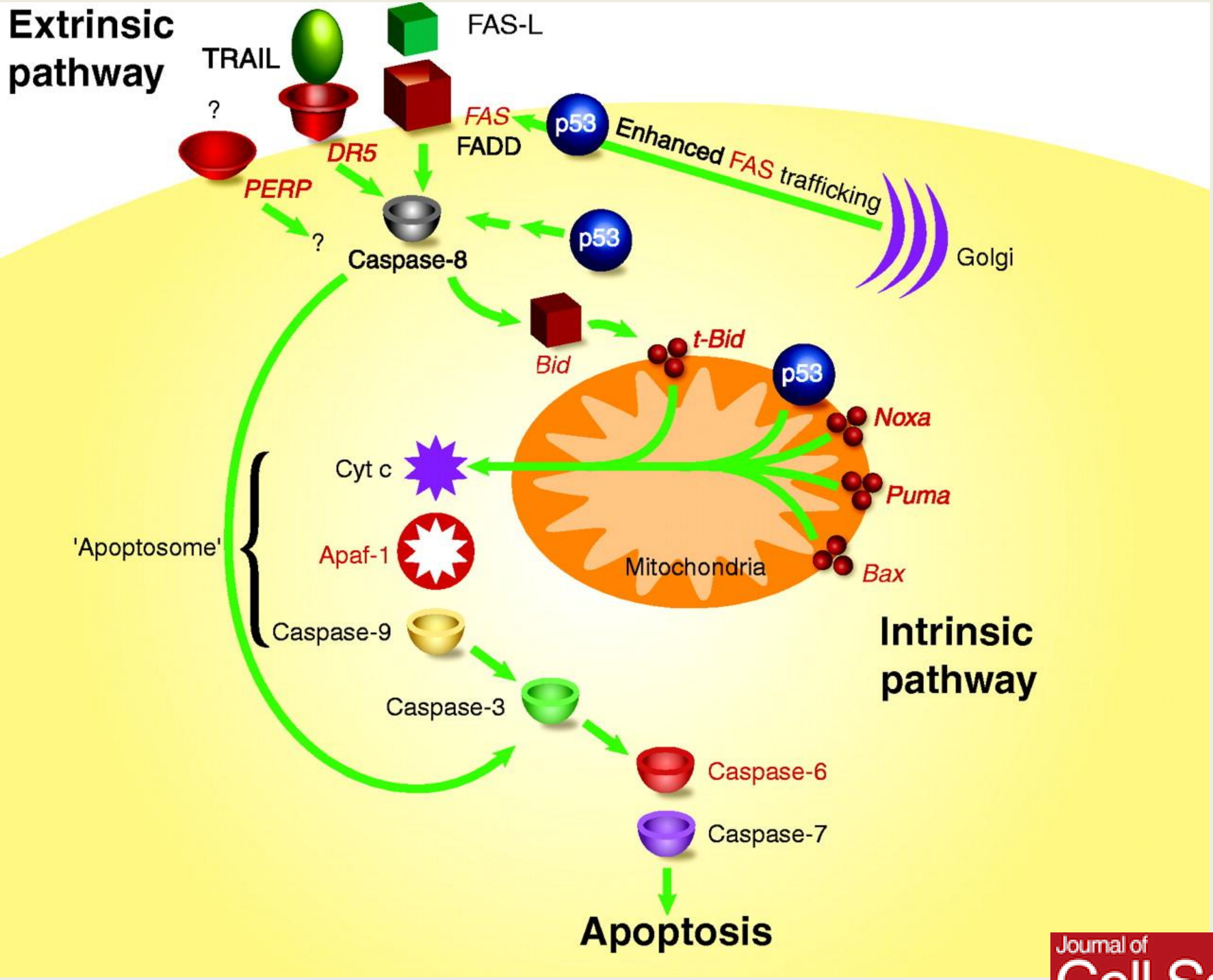
- **Bcl-2** interferes with Bax function by forming a hetero dimer with Bax. This leads to the closing of the channel and inhibition of cytochrome c translocation

Cytochrome c translocation initiates caspase cascade

- In the cytosol, cytochrome c binds to Apaf-1 to form apoptosome
- The apoptosome recruits procaspase 9 to generate the active caspase 9
- Caspase 9 then activates the executioner caspase 3, 6, 7



A model for p53-mediated apoptosis.



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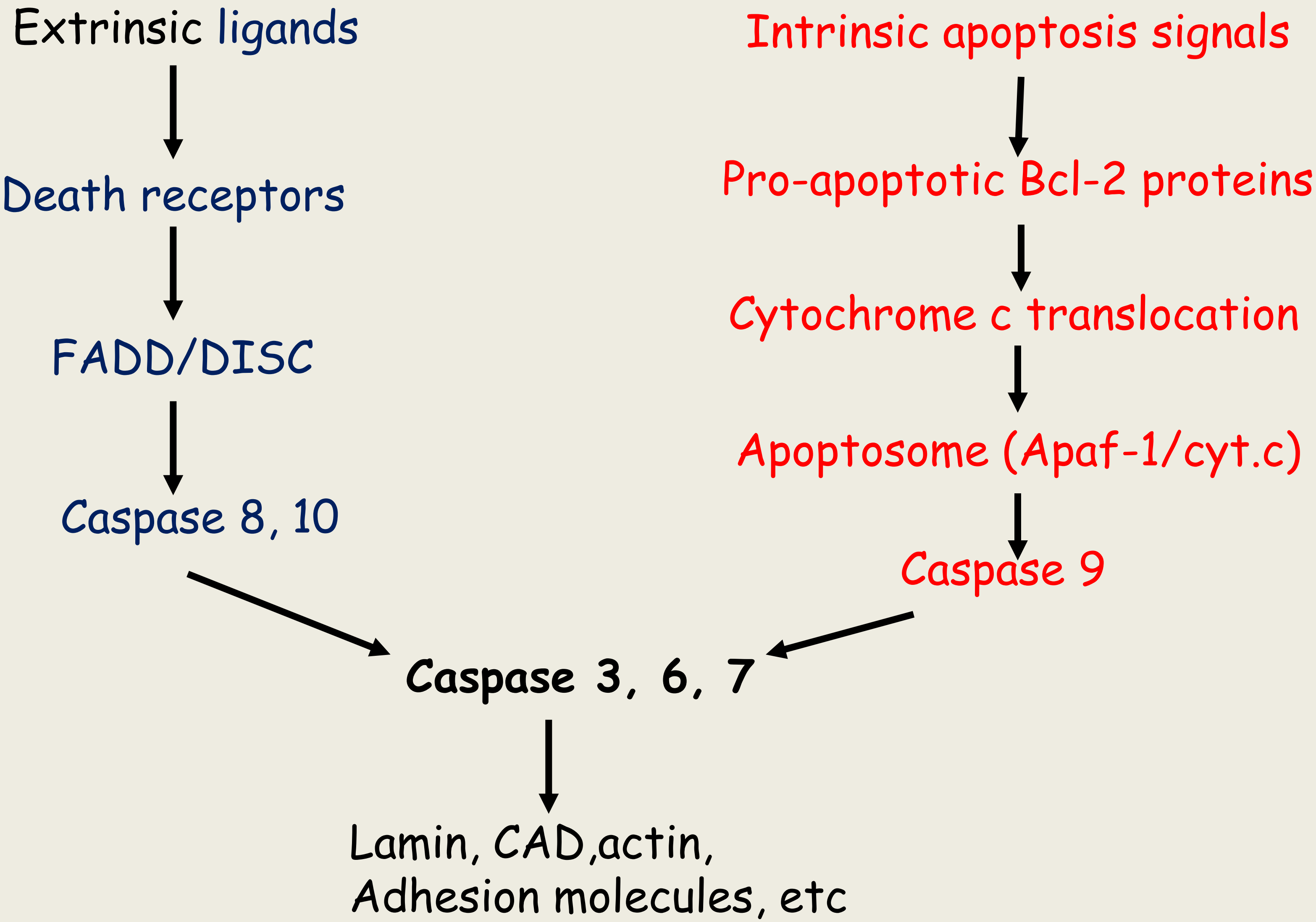
Haupt S et al. J Cell Sci 2003;116:4077-4085

Journal of Cell Science
jcs.biologists.org

Extrinsic and intrinsic pathways converge at executioner caspases



Extrinsic ligands

Death receptors

FADD/DISC

Caspase 8, 10

Intrinsic apoptosis signals

Pro-apoptotic Bcl-2 proteins

Cytochrome c translocation

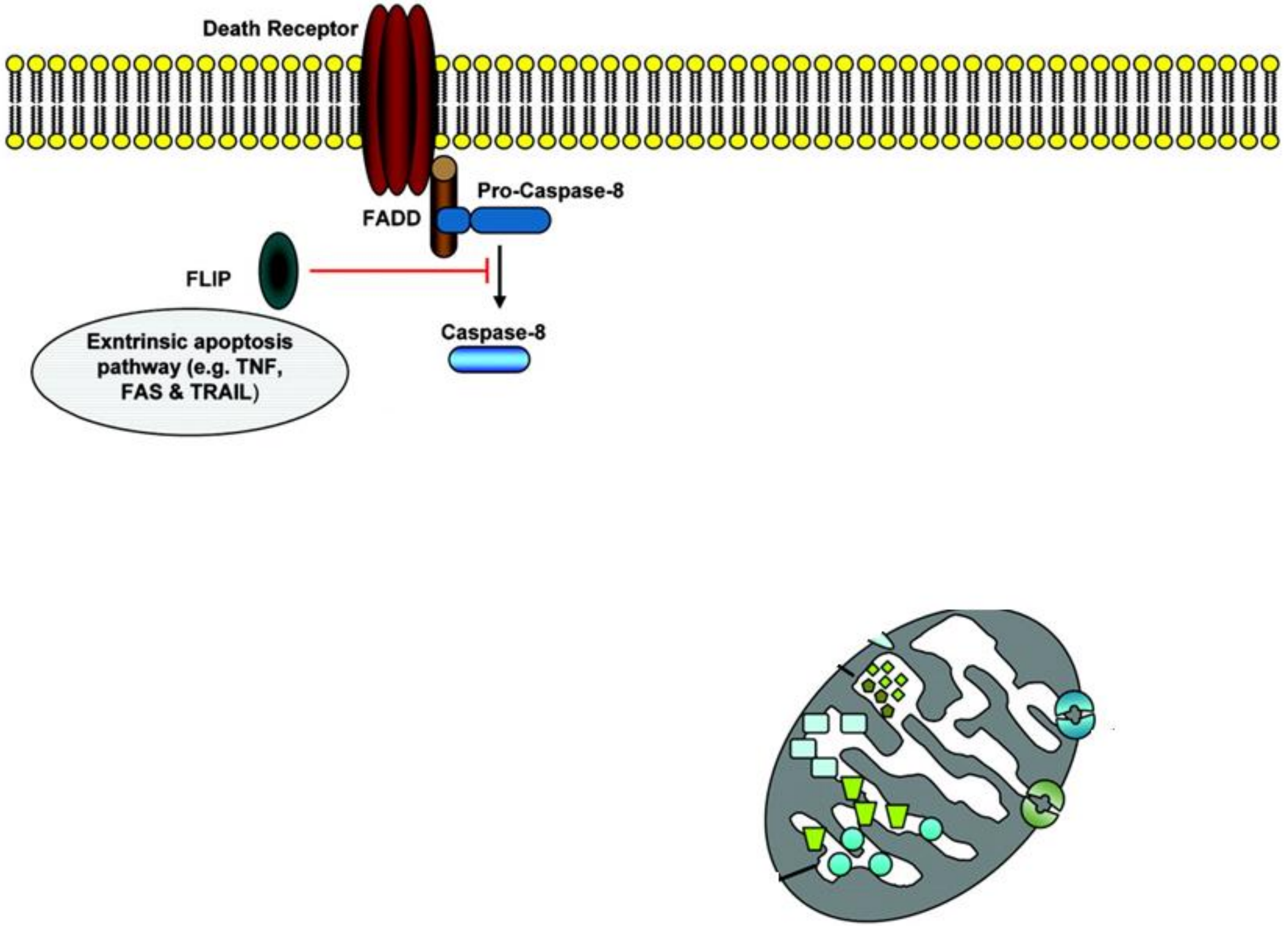
Apoptosome (Apaf-1/cyt.c)

Caspase 9

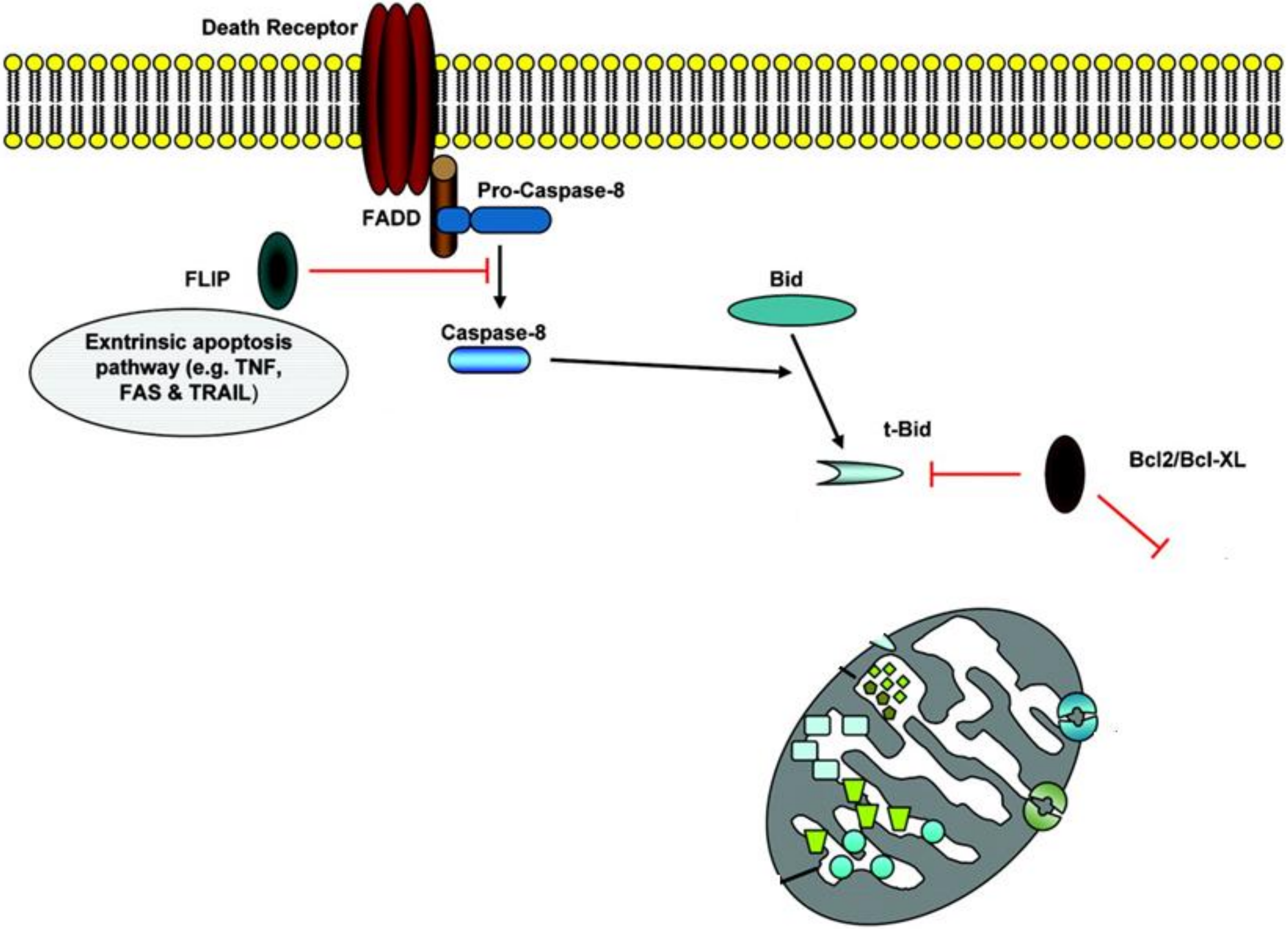
Caspase 3, 6, 7

Lamin, CAD,actin,
Adhesion molecules, etc

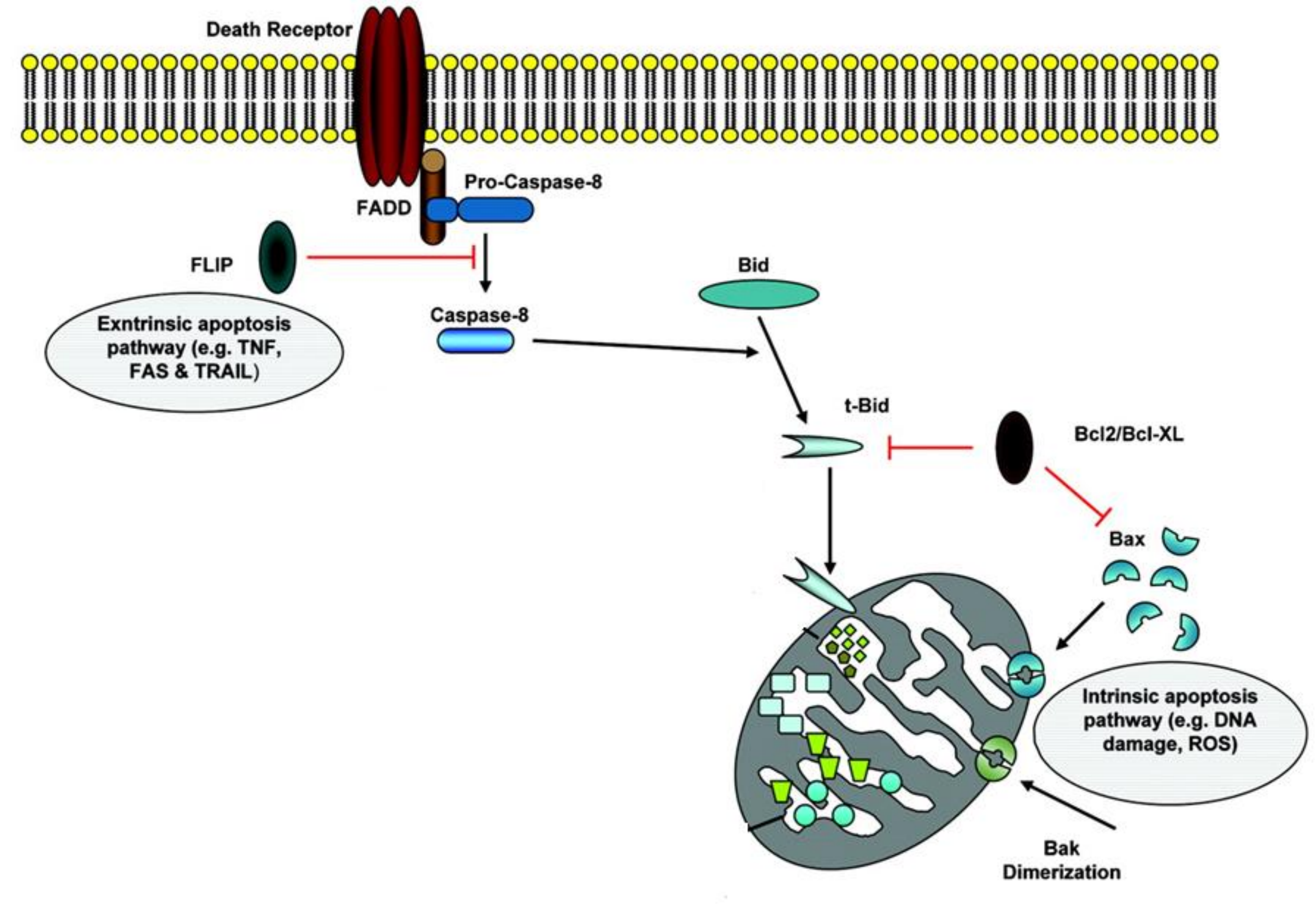
The molecular mechanisms of apoptosis.



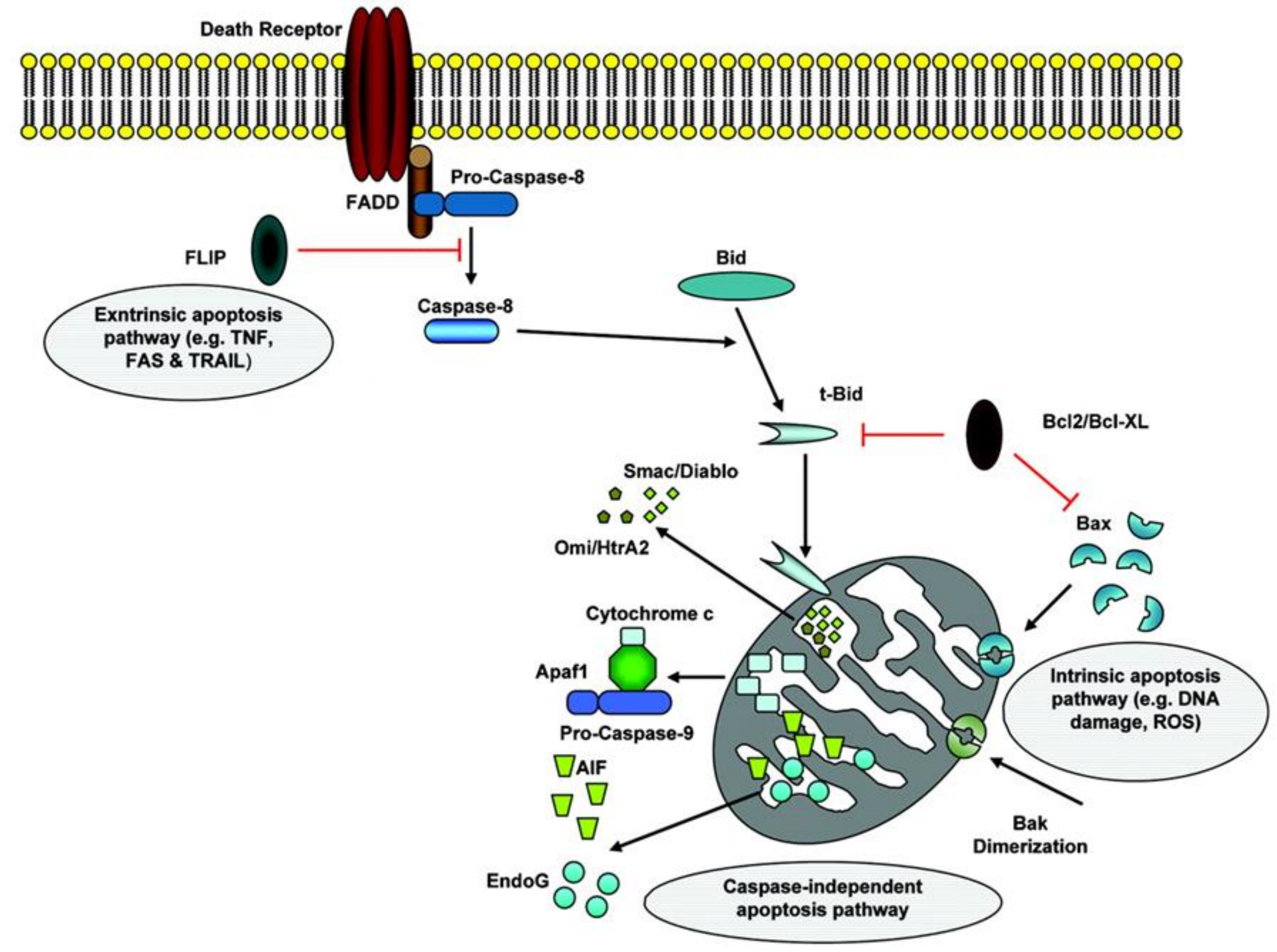
The molecular mechanisms of apoptosis.



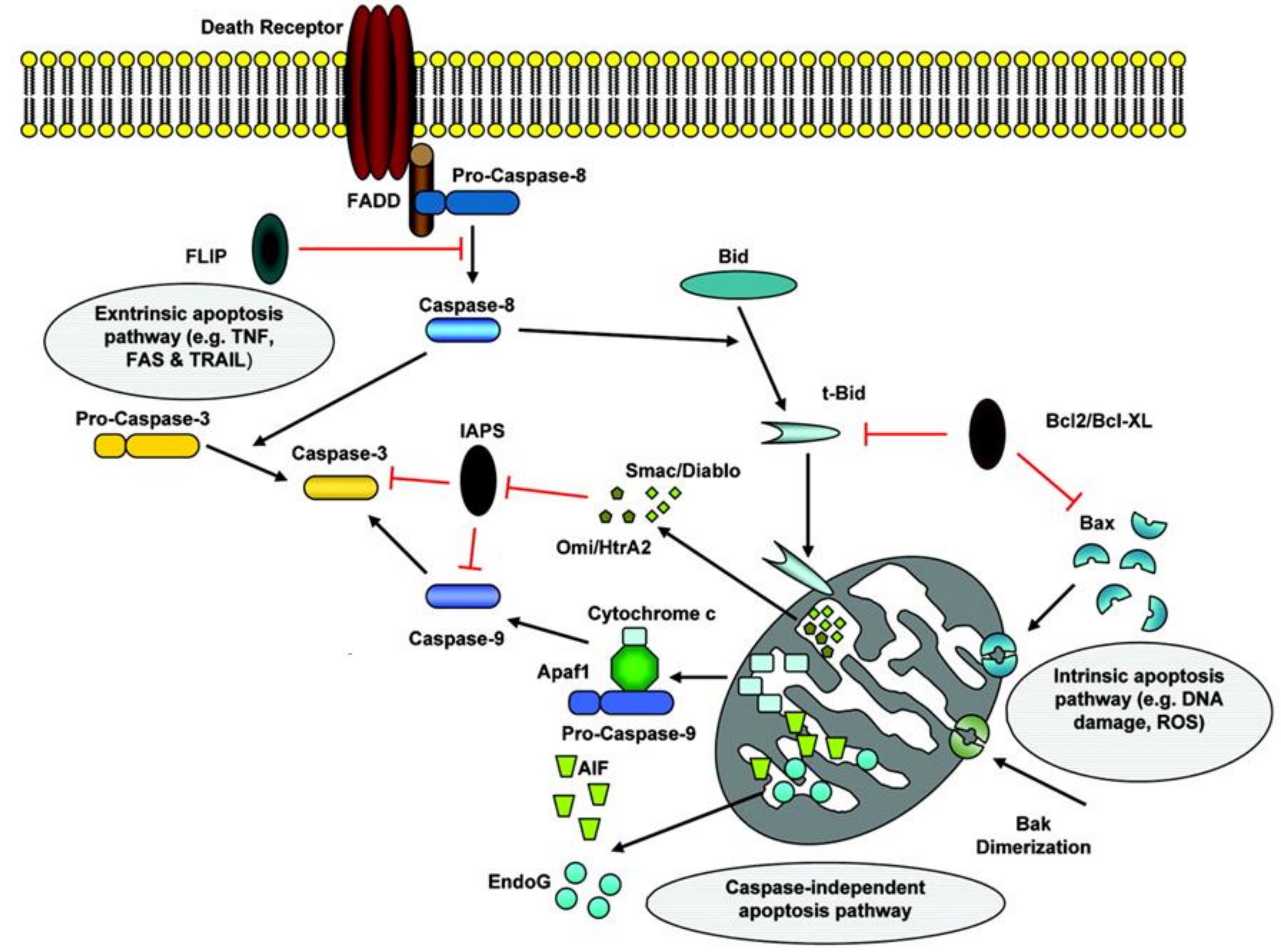
The molecular mechanisms of apoptosis.


Death Receptor
Pro-Caspase-8
FADD
FLIP
Exntrinsic apoptosis pathway (e.g. TNF, FAS & TRAIL)
Caspase-8
Bid
t-Bid
Bcl2/Bcl-XL
Bax
Intrinsic apoptosis pathway (e.g. DNA damage, ROS)
Bak Dimerization

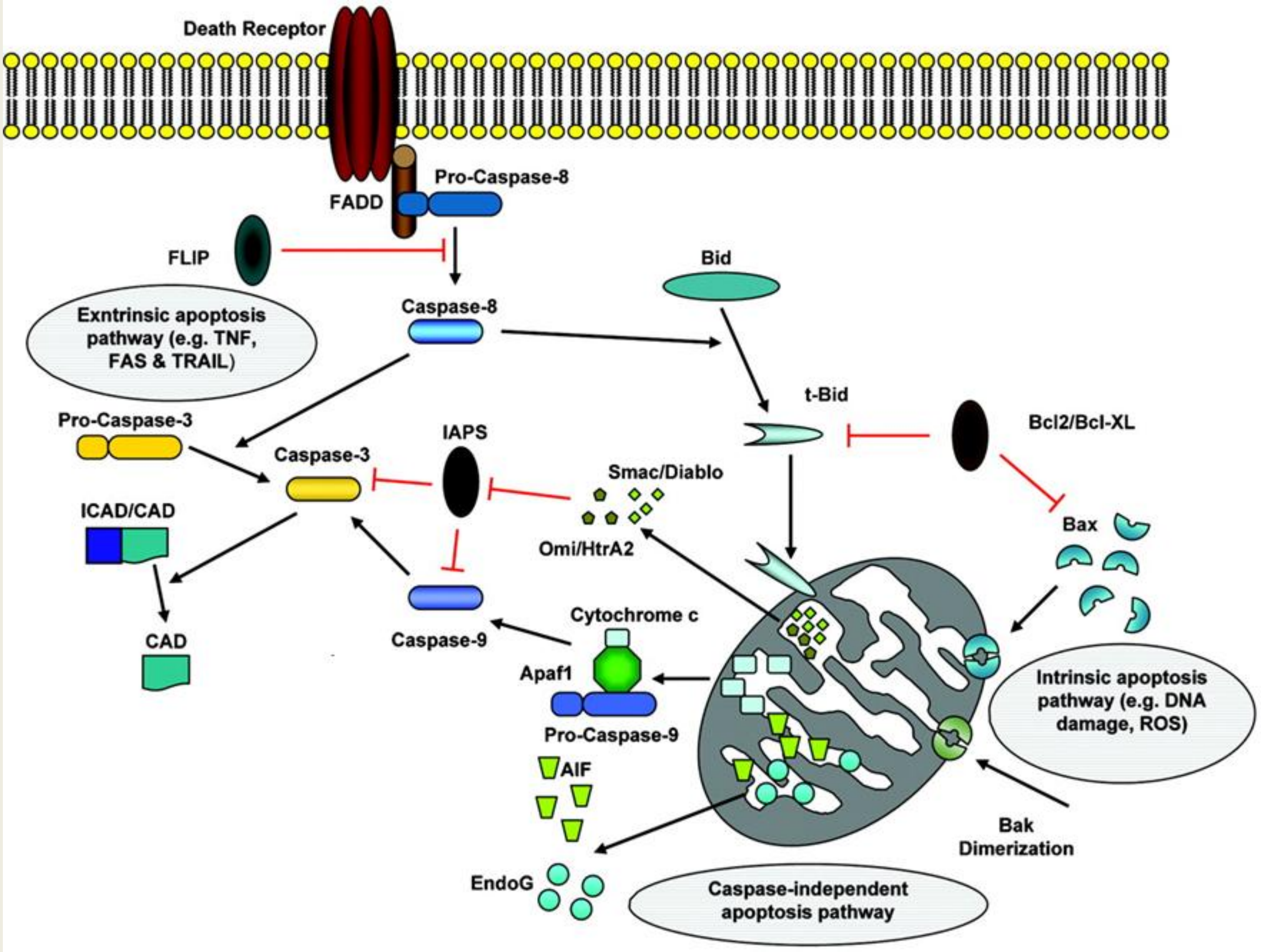
The molecular mechanisms of apoptosis.



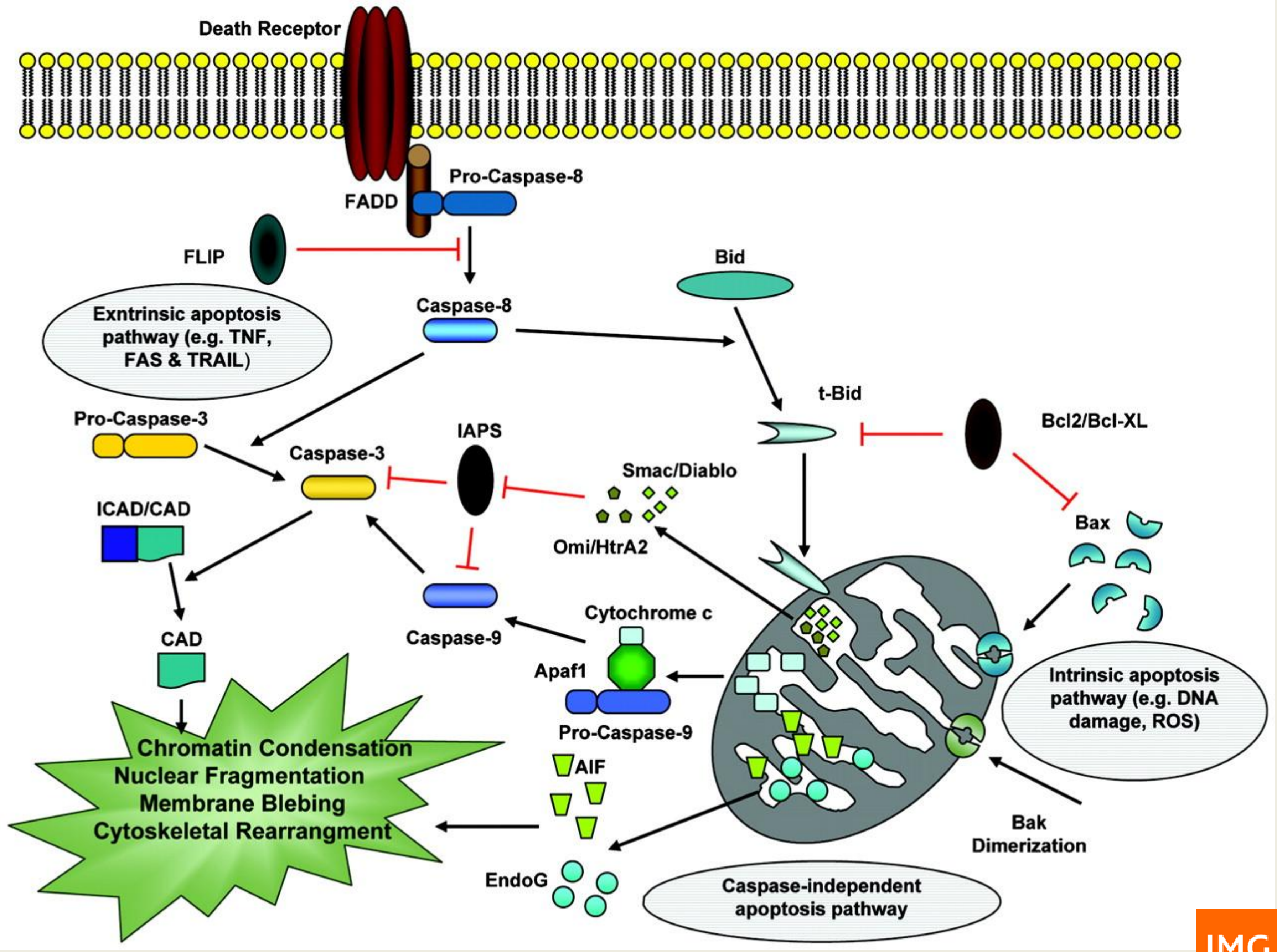
The molecular mechanisms of apoptosis.



The molecular mechanisms of apoptosis.



The molecular mechanisms of apoptosis.



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Ghavami S et al. J Med Genet 2009;46:497-510

Apoptosis integrates multiple cell-signaling pathways

- **Apoptosis** is programmed or controlled cell suicide
- Components of the cell are chopped up and packaged into vesicles that are digested by scavenger cells
- Apoptosis prevents enzymes from leaking out of a dying cell and damaging neighboring cells

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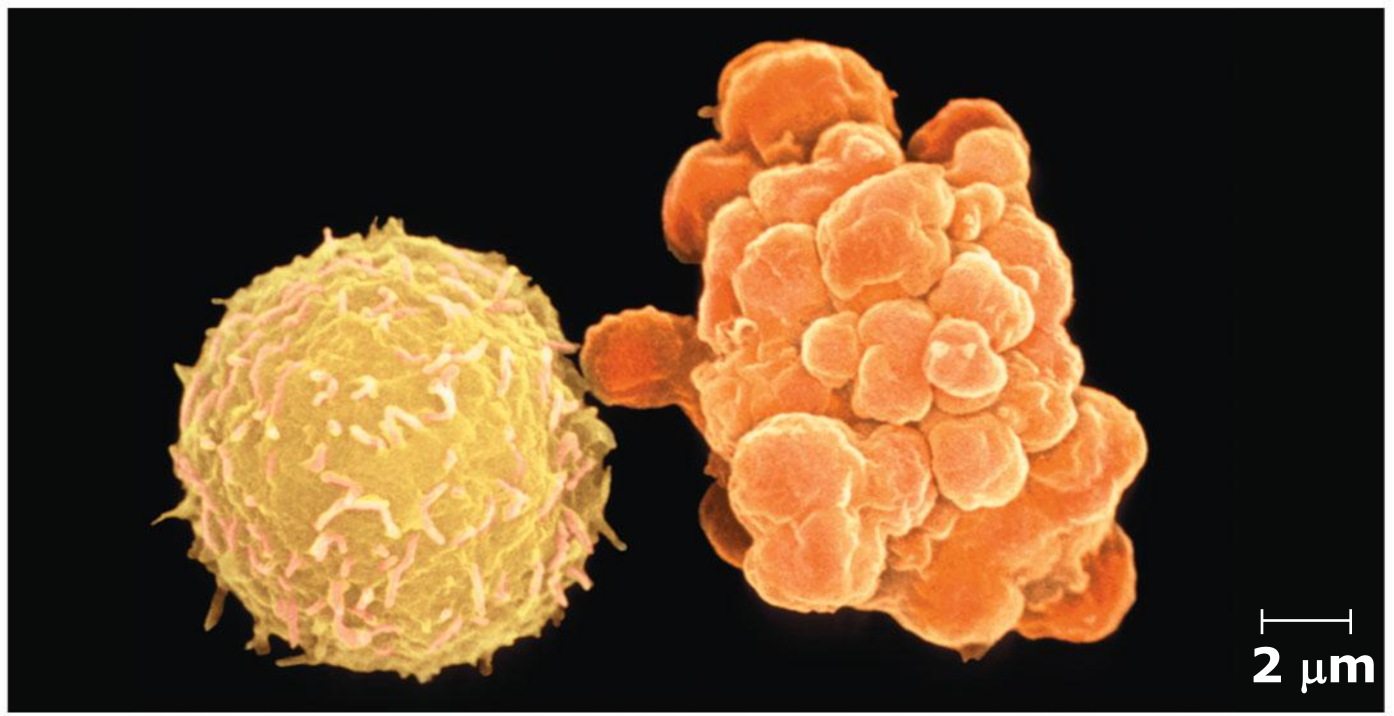
Figure 11.20



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Apoptosis in the Soil Worm *Caenorhabditis elegans*

- Apoptosis is important in shaping an organism during embryonic development
- The role of apoptosis in embryonic development was studied in *Caenorhabditis elegans*
- In *C. elegans*, apoptosis results when proteins that “accelerate” apoptosis override those that “put the brakes” on apoptosis

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Figure 11.21

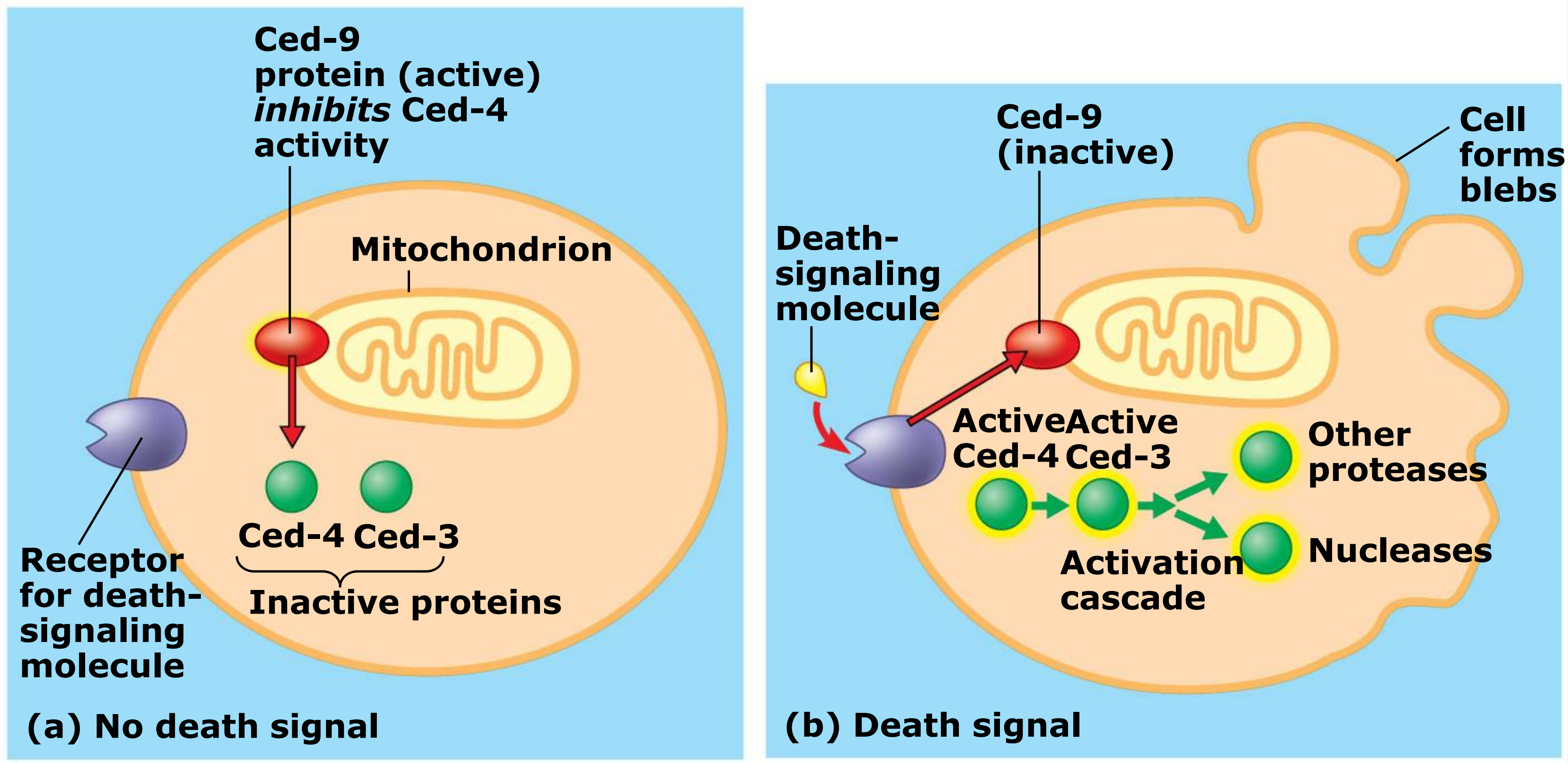

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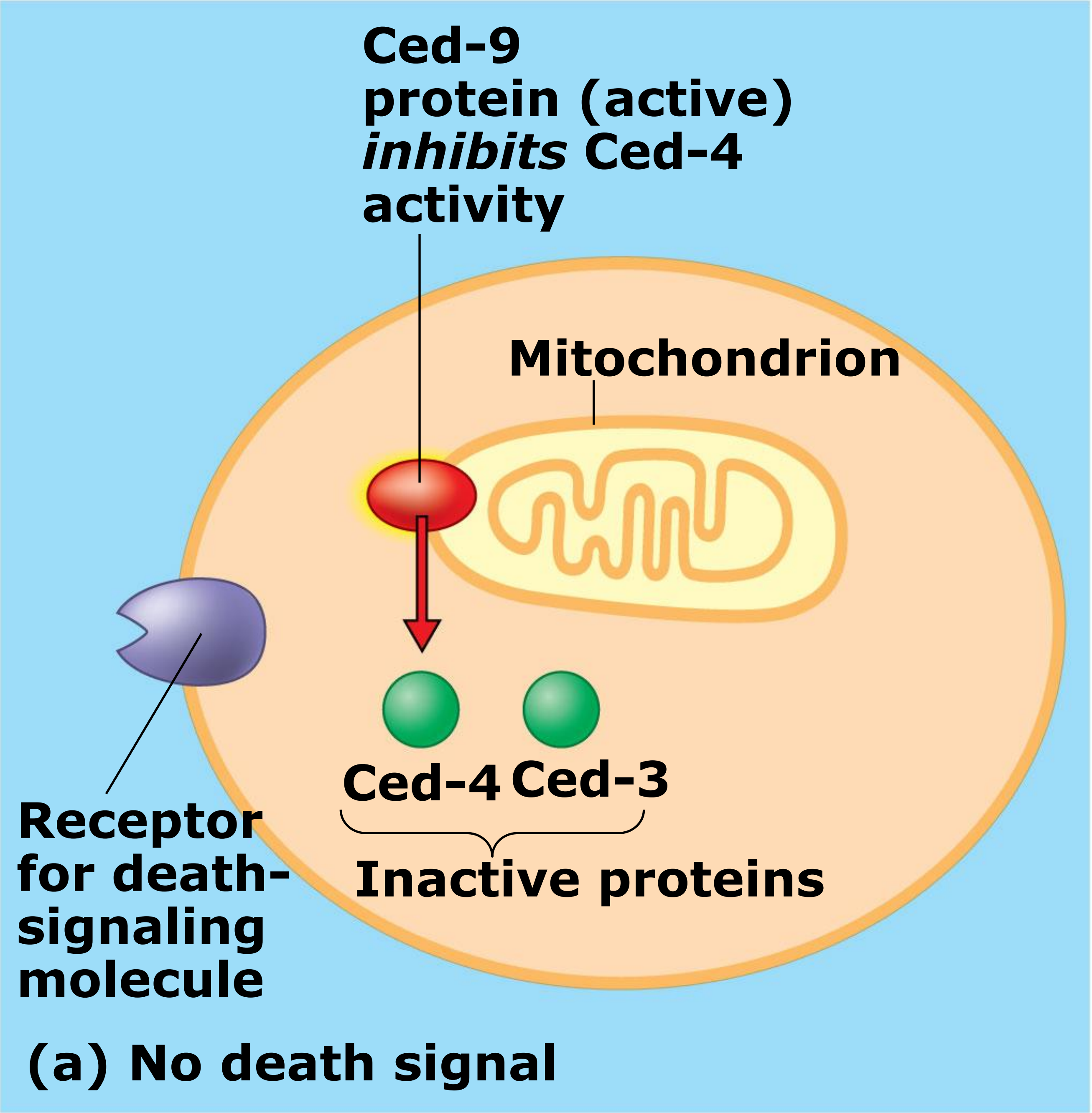
Figure 11.21a


Ced-9 protein (active) *inhibits* Ced-4 activity
Mitochondrion
Receptor for death-signaling molecule
Ced-4
Ced-3
Inactive proteins

(a) No death signal

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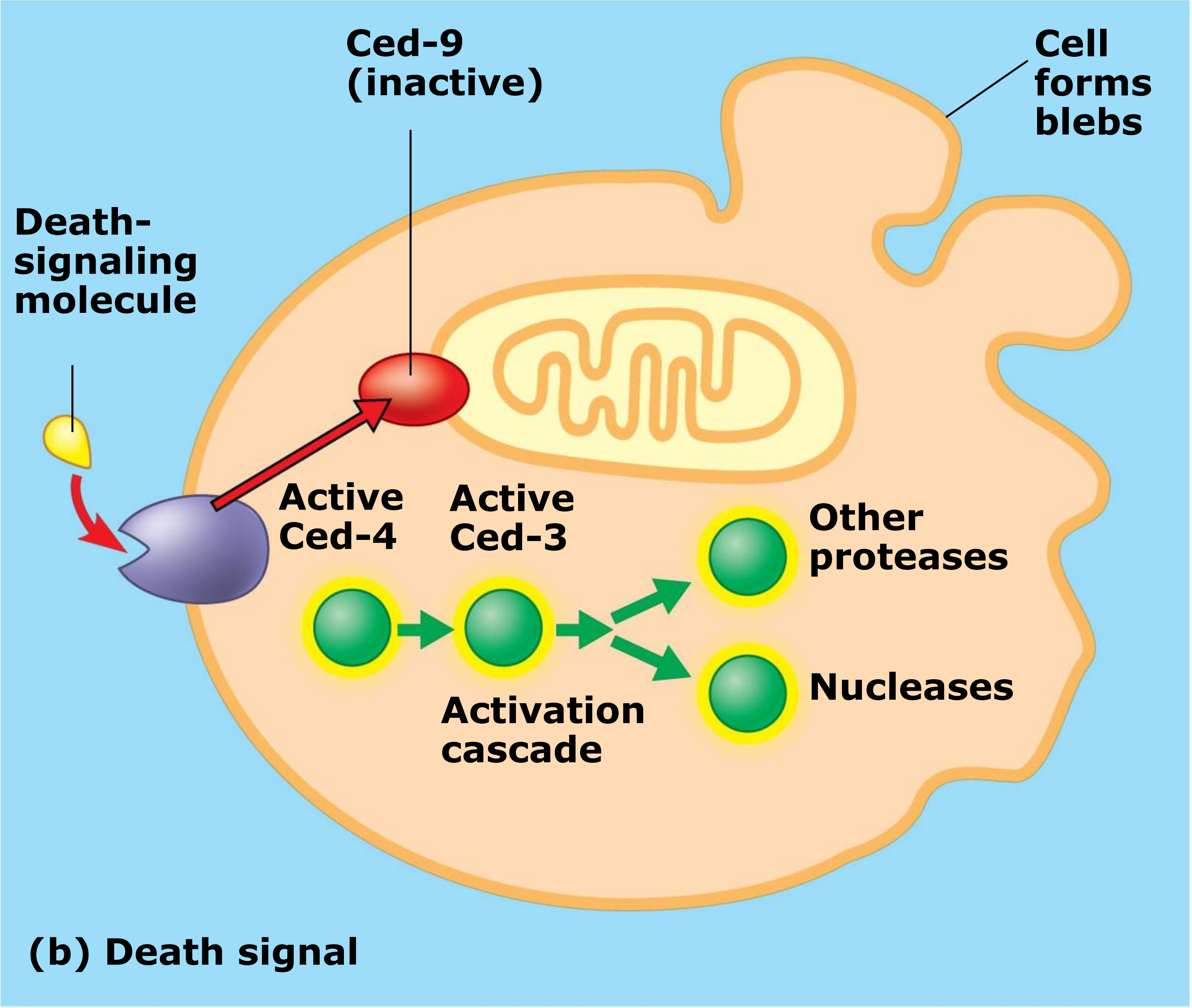
Figure 11.21b



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Apoptotic Pathways and the Signals That Trigger Them

- Caspases are the main proteases (enzymes that cut up proteins) that carry out apoptosis
- Apoptosis can be triggered by
  - An extracellular death-signaling ligand
  - DNA damage in the nucleus
  - Protein misfolding in the endoplasmic reticulum

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- Apoptosis evolved early in animal evolution and is essential for the development and maintenance of all animals
- Apoptosis may be involved in some diseases (for example, Parkinson’s and Alzheimer’s); interference with apoptosis may contribute to some cancers

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Figure 11.22

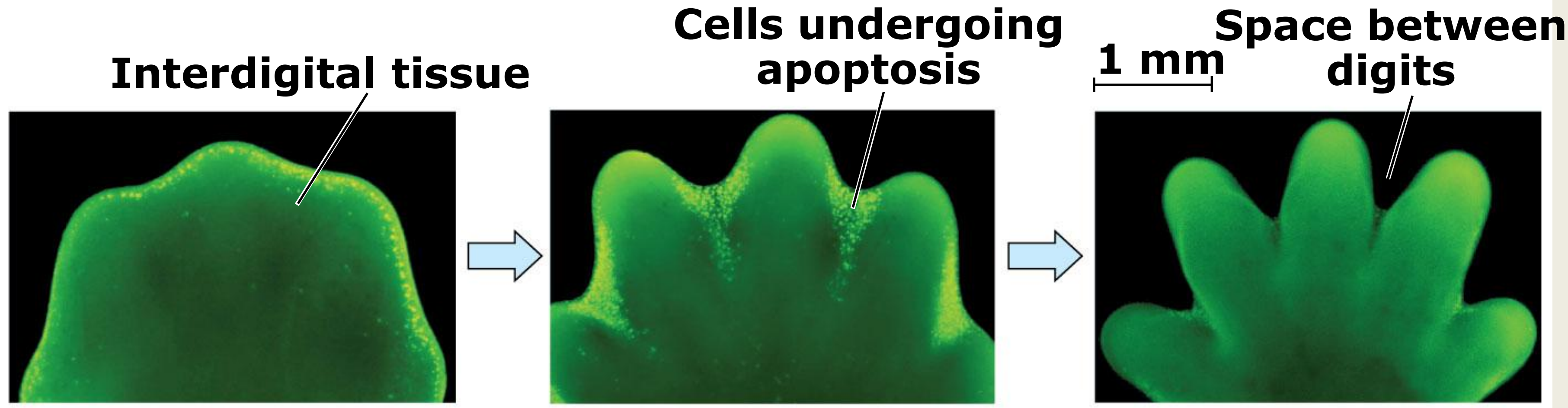

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Figure 11.22a

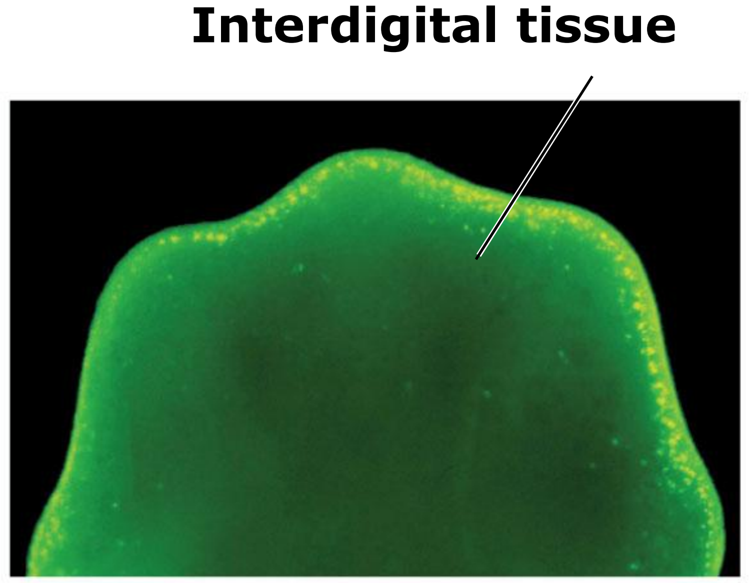

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Figure 11.22b

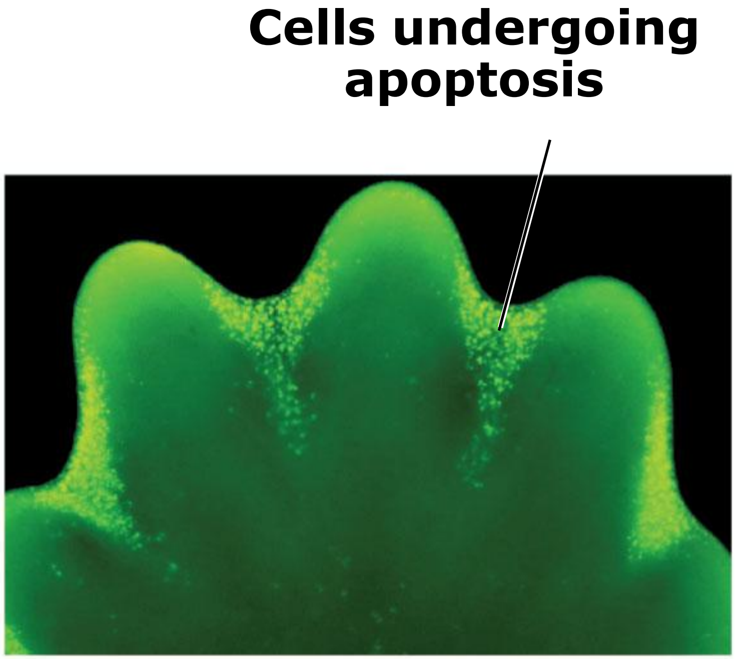

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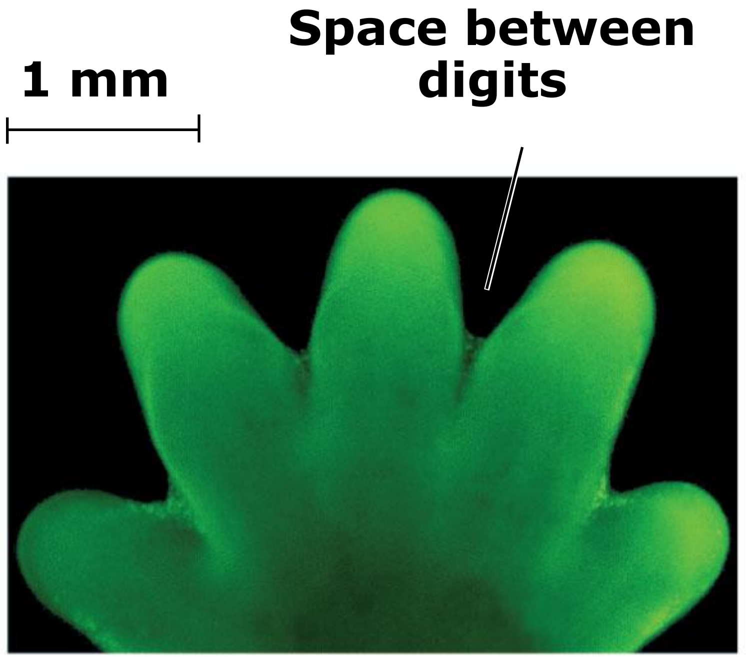
Figure 11.22c



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